

## Appendix F

### Standard Operating Procedures

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## STANDARD OPERATING PROCEDURE F1

### SURFACE SEDIMENT COLLECTION

#### A Introduction

Surface sediment samples (0- to 10-cm) will be collected from a boat or from land following processing will follow standardized procedures as described in Ecology's Sediment Cleanup User's Manual II (SCUM II) (Ecology 2017) and Puget Sound Estuary Program (PSEP). The SOPs for these procedures are described below.

#### B Sample Collection by Boat

The primary method for surface sediment sample collection will be to use a pneumatic grab sampler deployed from a sampling vessel. Surface sediment samples will be collected as described in the following steps:

1. Using a differential global positioning system (DGPS)<sup>1</sup> with sub-meter accuracy, maneuver the sampling vessel to the sampling location.
2. Open the decontaminated grab sampler jaws to the deployment position.
3. Guide the sampler overboard until it is clear of the vessel.
4. Using DGPS, position the sampling vessel such that the DGPS receiver (located on top of the sampling frame) is within 3 m (10 feet) of the target sampling location.
5. Lower the sampler through the water column to the bottom at a speed of approximately 0.3 m/s.
6. Record the DGPS location of the boat when the sampler reaches the bottom.
7. Record the water depth and time.
8. Retrieve the sampler, raising it at a speed of approximately 0.3 m/s.
9. Guide the sampler aboard the vessel and place it on the work stand on the deck, taking care to avoid jostling that might disturb the integrity of the sample.
10. Examine the sample using the following sediment acceptance criteria:
  - Sample contains sediment; samples that are predominately gravel, rock, or debris will be rejected.
  - Sediment is not extruding from the upper face of the sampler (indicating sampler was advanced deeper than the target penetration depth).

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<sup>1</sup> A Trimble® SPS461 or similar DGPS receiver unit will be employed for the various sampling methods outlined in this QAPP. The DGPS receiver will be calibrated daily to ensure that it is accurately recording positions from known benchmarks and functioning within the individual unit's factory specifications.

- Overlying water is present (indicating minimal leakage).
- Sediment surface is relatively flat (indicating minimal disturbance or winnowing).
- A penetration depth of at least 11 cm has been achieved.

If these sample acceptance criteria are not met, the sample will be rejected. If the initial attempt to collect a sample is not successful due to difficult substrate (e.g., presence of riprap or other debris), up to three subsequent attempts will be made within 10 m (32 feet) of the proposed location. If the initial attempt and three subsequent attempts do not result in a sample that meets the appropriate acceptance criteria, a different sampling location may be selected in consultation with EPA and LDWG.

After sample acceptance, the following observations will be noted in the field logbook or surface sediment collection form:

- Elevation of bed at sampling location
- DGPS location
- Depth as read by the boat's depth sounder and sample collection time
- Maximum penetration depth (nearest 0.5 cm)

## **C Sample Collection from Shore**

For intertidal locations that cannot be sampled from a boat and must be manually sampled from the shoreline during a lower tide, sediment will be collected by directly scooping sediment from the 0- to 10-cm depth with a clean, stainless steel spoon into a clean, stainless steel bowl.

The following observations will be noted in the field logbook or surface sediment collection form:

- Estimated elevation of bed at sampling location
- DGPS location
- Sample collection time

## **D Sample Processing**

After sediment collection and homogenization has been completed, the following steps will be completed to process the sediment samples:

1. **Record information** – Record information regarding the depth of the sample (generally 10 cm), sediment characteristics (e.g., color, smell, grain size, presence of debris, redox layer [if visible], etc.), and any necessary revisions to the sampling location or comments relative to sample quality, on the sediment collection forms. Take photographs of anything of note

and document any deviations from the approved sampling plan on a Protocol Modification Form (Appendix D-1).

2. **Collect and homogenize sample sediment** – The sediment at each location will be transferred directly from the grab sampler (or hole, if collected manually from shore) into a pre-cleaned stainless steel bowl or cauldron and stirred with a clean, dedicated, stainless steel spoon or spatula until texture and color homogeneity have been achieved (Ecology 2017). Any large non-sediment items, such as gravel, shells, wood chips, or organisms (e.g., clams), will be removed prior to homogenization.
3. **Dispense into jars** – Sediment will be dispensed into clean and labelled jars. If collected, sulfides and ammonia will be analyzed in all potential bioassay samples. These analyses will be expedited in order to have data available prior to the initiation of toxicity testing. Subsamples for sulfides and ammonia will be collected from the homogenized composite sample. The sulfide subsamples will be placed in a 4-oz jar with a Teflon® septa filled with zero headspace. The sample jar will contain 5 mL of 2 Normal zinc acetate per 30 g of sediment as a preservative. The sulfide sample will be placed in the jar, covered, and shaken vigorously to completely expose the sediment to the zinc acetate. The jar will be labeled to indicate that zinc acetate has been added and stored in the dark at 0 to 6°C.
4. **Label jars** – Sample labels will contain the project number, sampling personnel, date, time, and sample ID. A complete sample label will be affixed to each individual sample jar. Labels will be filled out as completely as possible prior to each sampling event.
5. **QC jars and forms** – Thoroughly check all sample containers for proper identification, analysis type, and lid tightness. The FC will be responsible for reviewing sediment sample information recorded on field forms (Appendix D) and will correct any improperly recorded information.
6. **Prepare for delivery to the analytical laboratory** – Pack each container carefully to prevent breakage and place inside a cooler with ice for storage at the proper temperature ( $\leq 4 \pm 2^{\circ}\text{C}$ ) for delivery to the analytical laboratory.

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## STANDARD OPERATING PROCEDURE F2

### SUBSURFACE SEDIMENT COLLECTION

#### **A Introduction**

Subsurface sediment core samples will be collected primarily collected from a sampling vessel (using a vibracorer), or will be manually from shore in intertidal areas where collect from a vessel is not possible. Procedures for these two collection options are described below.

#### **B Sample Collection by Boat**

##### ***B1 Collect Sediment***

When sampling from a boat, the vibracorer will be deployed from the sampling vessel using an A-frame with a hydraulic winch system. The vibracorer consists of a vibrating power head attached to a 4-in.-diameter core barrel (length to be dependent on the target core depth). Sediment core samples will be collected and processed according to the following procedures:

1. The sampling vessel will be maneuvered to the proposed sampling location.
2. The vibracorer and a decontaminated core tube will be deployed.
3. Continuous core samples will be collected to the project depth requirement or until refusal.
4. The depth of core penetration will be measured and recorded.
5. The sample core tube will be extracted, and the assembly will be retrieved aboard the vessel.
6. The core sample will be evaluated at the visible ends of the core tube to verify retention of the sediment in the core tube.
7. If the sediment core is acceptable (see criteria below), the core will be capped, labelled, and held vertically pending transfer to a processing crew.

Acceptance criteria for a sediment core sample are as follows:

- The material is collected to the target depth within the first three attempts.
- Recovery is at least 75% of the penetration depth.
- The core appears to be intact without obstructions or blocking.

If sample acceptance criteria are not achieved, the sample will be rejected. If repeated deployment (i.e., maximum three attempts) does not result in a sample that meets the acceptance criteria, a different sampling location may be selected based on consultation with EPA and LDWG.

Field forms and notes for all core samples will be maintained as samples are collected. The following information will be included in the sediment core collection forms and field notes:

- Water depth and tidal elevation (i.e., raw data), as well as the calculated mudline elevation of each sediment core location relative to MLLW
- Location of each sediment core as determined using a DGPS with sub-meter accuracy
- Date and time of collection for each sediment core
- Names of field supervisor and person(s) collecting and logging the sample
- Core penetration and recovery measurements
- Designation of each coring attempt as "accepted" or "rejected"
- Observations made during sample collection, including weather conditions, complications, ship traffic, and other details associated with the sampling effort
- Core location ID
- Take photographs of anything of note.
- Any deviations from the approved sampling plan on a Protocol Modification Form (Appendix D-1).

## **B2     *Process Core***

Sediment cores collected from a boat will be processed as soon as possible after a core has been collected that meets the acceptance criteria. The steps for processing the samples are as follows:

1. Prior to processing, evaluate the amount of compaction that may have occurred, and calculate the compaction correction factor (CCF) to be applied during core processing.
  - Measure the core depth (i.e., the compacted depth).
  - To calculate the CCF, divide the compacted depth by the penetration depth (i.e., the depth recorded during core collection and acceptance).
  - Example: If the core depth (i.e., compacted depth) at the time of processing is 2.83 feet (i.e., 2 feet 10 in.), and the core penetration depth (i.e., the core depth recorded at the time of collection) was 3.33 feet (i.e., 3 feet 3 in.), the CCF would be 0.85.
2. Carefully cut along the core tube liner to expose the sediment core for processing and photograph each core.
3. Record the description of each core on the sediment core processing form, including the following parameters, as appropriate, and take photographs of anything of note.

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- Core penetration depth (from the sediment core collection form)
  - Compacted core depth and calculated CCF
  - Corrected sample depth interval for each sample
  - Odor (e.g., hydrogen sulfide, petroleum)
  - Vegetation
  - Debris
  - Biological activity (e.g., detritus, shells, tubes, bioturbation, live or dead organisms)
  - Presence of oil sheen
  - Any other distinguishing characteristics or features.
4. For each core, separate the material from each target depth interval, applying (i.e., multiplying) the CCF to the target sample depth that will constitute the sample for laboratory analysis. For example, if the CCF for a subtidal sediment core is 0.85, the sample material to collect for a 0- to 60-cm analysis will come from the 0- to 51-cm interval (i.e.,  $60 \text{ cm} \times 0.85 = 51 \text{ cm}$ ).
  5. Transfer sample into a separate stainless steel bowl for homogenization.
    - For intertidal sediment cores, the target sample depth interval is 0- to 45- cm.
    - For subtidal sediment cores, the target sample depth interval is 0- to 60- cm.
    - For shoaling areas, the target sample depth interval is dependent on the thickness of the shoaled material (see Appendix G for estimated shoal depths).
    - For samples in the FNC, 1-foot (30-cm) Z-samples will also be collected in shoaling areas and at locations with elevations shallower than -15 feet MLLW.
  6. Homogenize the sediment until texture and color homogeneity have been achieved, removing large non-sediment items such as gravel, shells, wood chips, or organisms (e.g., clams) (Ecology 2017).
  7. Dispense sediment into labeled sample containers. Sample labels will contain the project number, sampling personnel, date, time, and sample ID. A complete sample label will be affixed to each individual sample jar. Labels will be filled out as completely as possible prior to each sampling event.
  8. Thoroughly check all sample containers for proper identification, analysis type, and lid tightness. The FC will be responsible for reviewing sediment sample information recorded on field forms (Appendix D) and will correct any improperly recorded information.
  9. Pack each container carefully to prevent breakage and place inside a cooler with ice for storage at the proper temperature ( $\leq 4 \pm 2^{\circ}\text{C}$ ) for delivery to the analytical laboratory.

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## **C Sample Collection from Shore**

If an intertidal sediment 0- to 45- cm core cannot be collected from the boat due to site access conditions (e.g., too shallow), then the core may be manually collected from shore during a lower tide. At the discretion of the field crew, one of the following two sampling options will be used, whichever is most suitable to the sampling location conditions. In addition, the field crew may use a combined or hybrid approach of the two methods, if necessary.

### **C1 Option 1: Use Shovel to Dig 45-cm-Deep Hole**

The first sampling option is to dig a hole using a shovel and collect the sample directly from the sidewall of the hole. The process for this option is as follows.

1. **Dig hole** – Using a transplanting spade (i.e., a shovel with a narrow blade), dig a 45-cm-deep hole at the identified location. If it is not possible to reach a depth of 45 cm within three attempts, the deepest hole among the attempts will be sampled using the methodology described below, and the depth of refusal will be recorded on the sediment core collection form. At least one side of the hole should be approximately vertical to allow for the collection of the sample. Record any necessary revisions of the sampling location.
2. **Prepare for sampling** – Divide the vertical extent of the hole into three 15-cm sections (i.e., the bottom section 30 to 45 cm below the surface, the middle section 15 to 30 cm below the surface, and the top section from the surface down to 15 cm). If possible, use a spoon to draw a line in the sidewall of the hole at these breakpoints. The bottom section will be sampled first to ensure that the sample is collected prior to the hole filling with water.
3. **Collect and homogenize sample** – Collect the same amount of sediment from each of the three 15-cm subsections along the vertical extent of the hole, sufficient to fill a 16-oz stainless steel measuring cup. When filling the measuring cup (as described in steps 3a-3c), exclude any debris larger than approximately 5 mm in width. If differences pertaining to the diameter of the hole are apparent (e.g., the presence of differently colored material), the resulting sample should proportionally represent all material in the hole.
  - a. Starting with the bottom section of the hole (i.e., 45 to 30 cm), use a small, clean, stainless steel spoon to carefully collect an even amount of sediment from the sidewall by scraping the sidewall from the bottom of the hole to the marked 30-cm line. Fill the 16-oz measuring cup using this method, and dispense the contents into a large, stainless steel bowl.

- b. Repeat process in the middle section of the hole (i.e., scrape the sidewall from the 30-cm to the 15-cm line) to fill the measuring cup, and dispense the contents into the bowl containing the sediment from step 3a.
- c. Repeat process in the top third of the hole (i.e., 15 cm to the surface) to again fill the measuring cup, making sure to capture the full extent of this layer, including the surface material. Dispense the contents into the bowl containing the sediment from steps 3a and 3b.
- d. Homogenize the contents of the bowl with a stainless steel spoon until texture and color homogeneity have been achieved, and dispense the contents into clean and labelled jars.

The procedures for processing shore-collected cores are presented below.

### **C2     *Option 2: Use Hand-core Tube to Collect 45-cm Core***

The second sampling option is to use a hand-core tube to collect a 45-cm core, extrude the core, and then collect the sample from the interior of the core. This process for this option is as follows:

1. **Collect core** – Drive the pre-cleaned hand-core tube (internal diameter of 7 cm) into the sediment to a depth of 45 cm at the identified location, or as near as possible based on the substrate and debris. Cap the top of the tube and pull core out of the sediment. If it is not possible to reach a depth of 45 cm on the first attempt, up to three attempts should be made in that area (initial attempts will be retained in the core tube or extruded onto a piece of foil). After the third attempt, sample the deepest core using the methodology described below, and record the depth of refusal on the surface sediment collection form (Appendix D-1). Record any necessary movement of the sampling location.
2. **Collect and homogenize sample** – Extrude the contents of the core into a pre-cleaned stainless steel bowl and homogenize with a clean, stainless steel spoon until texture and color homogeneity have been achieved. Any debris wider than approximately 5 mm will be discarded.

The procedures for processing shore-collected cores are presented below.

### **C3     *Processing Cores Collected from Shore***

After sediment collection and homogenization has been completed, the following steps will be completed to process the sediment cores:

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1. **Record information** – Record information regarding the depth of the core (generally 45 cm), sediment characteristics (e.g., color, smell, grain size, presence of debris, etc.), and necessary revisions to the sampling location on the sediment core collection and processing forms. Take photographs of anything of note and document any deviations from the approved sampling plan on a Protocol Modification Form (Appendix D-1).
  2. **Dispense into jars** – Sediment will be dispensed into clean and labelled jars. Sample labels will contain the project number, sampling personnel, date, time, and sample ID. A complete sample label will be affixed to each individual sample jar. Labels will be filled out as completely as possible prior to each sampling event.
  3. **QC jars and forms** – Thoroughly check all sample containers for proper identification, analysis type, and lid tightness. The FC will be responsible for reviewing sediment sample information recorded on field forms (Appendix D) and will correct any improperly recorded information.
  4. **Prepare for delivery to the analytical laboratory** – Pack each container carefully to prevent breakage and place inside a cooler with ice for storage at the proper temperature ( $\leq 4 \pm 2^{\circ}\text{C}$ ) for delivery to the analytical laboratory.

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**Title: Determining Wet-Sieve Grain Size of Sediment**

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## 1.0 SCOPE

This protocol provides a gross field or laboratory measurement of percent fines in a sediment sample.

## 2.0 SUMMARY OF PROCEDURE

Field collected material from a given sample location is sieved using a USA Standard Testing Sieve #230 (63µm) to determine volume of fines. The volume retained in the sieve, subtracted from the original aliquot, provides the volume of fines.

## 3.0 EQUIPMENT

- Modified ponar grab with line of an appropriate length and accessories. Includes any needed shackles or swivels.
- USA Standard Testing Sieve #230 (63µm)
- 50-mL measuring cup
- 100-mL graduated cylinder
- Small plastic funnel
- Plastic spoon
- Squirt bottle filled with water (site water or deionized water)
- A pump-action compression garden sprayer (1 to 2 gallon size)
- Safety glasses or goggles and chemical-resistant gloves (if contamination is suspected to be present)

## 4.0 PROCEDURE

- 1 Thoroughly rinse the sieve and all other equipment to ensure that no sediment or other detritus is present.
- 2 Collect a homogenized sediment aliquot in a 50-mL cup, removing excess sediment from the top and side of the cup.
- 3 Transfer the 50-mL sediment aliquot from the cup to the sieve using the spoon. Thoroughly rinse the cup and spoon into the sieve with water to ensure the entire aliquot has been transferred.
- 4 Gentle rinse the sieve with running water until the stream of water coming from the bottom of the sieve is clear. Rinse the remaining sediment to one side of the sieve.

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- 5 Place a plastic funnel into the 100-mL graduated cylinder and position the lip of the sieve over the funnel. Using the squirt bottle, rinse the retained sediment into the graduated cylinder, directing the stream of water through the back of the sieve. Continue rinsing until all the sediment has been transferred to the graduated cylinder. If needed, rinse any sediment that may have adhered to the funnel. The rinse water should not overflow the graduated cylinder. If it appears the graduated cylinder will overflow before all sediment has been transferred, discard the sample and repeat the entire procedure.
- 6 Allow the sediment to settle completely in the graduated cylinder (~1-2 minutes) and record the amount of sediment present. This measurement represents the ***volume retained***. Also record any turbidity observed in the overlying water.
- 7 The ***volume retained*** (in mL), subtracted from the original 50-mL aliquot, provides the volume that passed through the sieve, or ***volume of fines***, in the 50-mL sample. Multiplying this remainder by two gives the volume of fines in 100-mL or ***percent fines***. The formula can be stated as:  
***Percent fines = (50-mL – Volume Retained in mL) × 2.***

## 6.0 SAFETY

The standard safety considerations for aquatic sampling - caution deploying and retrieving equipment, utilizing proper clothing and safety gear, and stepping in the bight of lines or cables - apply to the field crew during sampling. The designated safety officer on the vessel shall be responsible for ensuring the safety of personnel and will be contacted immediately in the event of an accident or emergency. Particular care should be given to fingers and hands when working with any sampling device. The sediment, soil, and water samples may contain contaminants. Care should be taken to avoid contact with the samples. Protective gloves, glasses and proper clothing should be worn. Waste sample material should be properly disposed.

## 7.0 PERSONNEL

Persons who will perform this procedure should first read this SOP and then operate under the supervision of an experienced individual for at least one series of grain size determinations.

## 8.0 REFERENCES

PSEP. 1997. Recommended Guidelines for Sampling Marine Sediment, Water Column, and Tissue in Puget Sound. Puget Sound Estuary Program. Sampling Chapter. April 1997.

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**Title: 10-Day Acute Sediment Toxicity Test with Marine Amphipods (PSEP)**


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## 1.0 SCOPE

This test determines the short term, adverse effects of potentially contaminated sediment on marine amphipods. Sediment toxicity testing will be conducted according to procedures outlined in **Recommended Guidelines for Conducting Laboratory Bioassays on Puget Sound Sediments (1995)**, including modifications from the Sediment Management Annual Review Meeting (SMARM) clarification papers. Other references include **USACE/USEPA (1991) (OTM)** and **USACE/USEPA (1998) (ITM)** (for dredged sediments), **ASTM E 1367**, and **EPA/600/R-94/025**.

## 2.0 SUMMARY OF TEST

### 2.1 Approach

**Table 1. Conditions for Performing 10-Day Solid Phase Toxicity Testing on Marine Amphipods**

Test type	Static Non-renewal*
Test duration	10 Day
Lighting	Ambient and Constant
Test chamber size	1-L glass beaker
Test sediment depth	2 cm (~175 mL)
Test solution volume	775 mL (Chamber Vol. up to 950 mL)
Renewal of test solution	None*
No. of organisms per chamber	20
No. of replicates per treatment	5 test replicates 2 sacrificial chambers (one being the water quality surrogate) recommended minimum
Feeding	None
Test solution aeration	Trickle-flow (sufficient to maintain DO levels above 60% saturation)

\* Static renewal, intermittent flow or continuous flow tests may be used where it is necessary to maintain water quality parameters, e.g., dissolved oxygen (DO) or ammonia.

**Title: 10-Day Acute Sediment Toxicity Test with Marine Amphipods (PSEP)**

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## 2.2 Physical Requirements

**Table 2. Species Specific Test Condition Summaries**

Species	<i>Ampelisca abdita</i>	<i>Rhepoxynius abronius</i>	<i>Eohaustorius estuaries</i>	<i>Leptocheirus plumulosus</i> <sup>5</sup>
Life Stage Tested	Immature amphipods	Mature amphipods 3-5 mm, mixed sexes	Mature amphipods 3-5 mm, mixed sexes	Mature amphipods 2-4 mm, mixed sexes
Feeding	Will not be fed	Will not be fed	Will not be fed	Will not be fed
Temperature (°C)	20 ± 1	15 ± 1	15 ± 1	25 ± 2
Salinity (ppt)	28 ± 1	28 ± 1	28 ± 1 or ambient <sup>4</sup>	20 ± 2
pH	7-9	7-9	7-9	7-9
DO (≥ 60% Saturation)	4.6 mg/L	5.1 mg/L	5.1 mg/L	4.4 mg/L
Grain Size <sup>1</sup>	> 60% fines (> 20% clay fraction) <sup>2</sup>	< 60% fines	0 – 99.4% silt-clay; Provided clay fraction < 20% <sup>2</sup>	< 70% fines, <70% sand
Total Ammonia (mg/L, pH 7.7)	< 30	< 30	< 60	< 60
Un-ionized Ammonia (mg/L, pH 7.7)	< 0.4	< 0.4	< 0.8	< 0.8
Sulfides	N/A <sup>3</sup>	N/A <sup>3</sup>	N/A <sup>3</sup>	N/A <sup>3</sup>

<sup>1</sup> Grain size distributions are recommended guidelines and should not be considered absolute criteria. Species selection generally includes discussion with regulatory agencies and share holders and can be chosen exclusive from grain size characteristics (i.e. comparison to historical data with same species, species availability, etc.)

<sup>2</sup> SMARM clarification paper: 10/20/99

<sup>3</sup> Specific guidance for sulfide sensitivities have not been well established.

<sup>4</sup> Test salinity for *E. estuarius* may be conducted at the interstitial salinity (ambient) of the test sediments. The target test salinity should be approved by the client or regulatory agency, and will vary agency depending upon the objectives of the testing program.

<sup>5</sup> Direct guidance for *L. plumulosus* is not given under PSEP guidelines; however, test conditions are similar to that of *E. estuarius* and described in other guidance documents (EPA 1991, 1994).

## 3.0 TEST ORGANISM

The test organism should be selected based on availability, sensitivity to test materials, tolerance to ecological conditions, ecological importance, and ease of handling in the laboratory. Ideally, organisms with wide geographical distribution should be selected so test results can be compared among laboratories with similar organisms. Test conditions for each amphipod species are summarized in Tables 1 and 2.

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**Table 3. Test Organism Suppliers**

Species	<i>Ampelisca abdita</i>	<i>Rhepoxynius abronius</i>	<i>Eohaustorius estuaries</i>	<i>Leptocheirus plumulosus</i>
Life Stage Tested	Immature amphipods, or mature females only	Mature amphipods 3-5 mm, mixed sexes	Mature amphipods 3-5 mm, mixed sexes	Mature amphipods 3-5 mm, mixed sexes
Sources	John Brezina and Associates, Dillon Beach, CA; Aquatic Research Organisms, Hampton, NH;	Doug Henderson, Puget Sound Organisms; John Brezina and Associates, Dillon Beach, CA	Northwest Aquatic Sciences, Newport, OR	Aquatic BioSystems, Fort Collins, CO; Aquatic Research Organisms, Hampton, NH;

### 3.1 Test Organism Care

Records will be kept, including the date and location collected, feeding regime, and sediment characteristics.

Holding time for amphipods is standardized to between 2 and 10 days.

### 4.0 TEST SUBSTANCE

The test sediments will be labeled, properly stored, and tracked by internal chain-of-custody procedures throughout its tenure at the facility. The sediments will not be heated, filtered, distilled, frozen, or otherwise altered without prior written consent by the Client. The test substance is stored at 0 – 6 °C in the dark, in a secure and distinct storage area. Containers should also have as little air as possible over the sediment or be stored with nitrogen gas in the overlying head space.

Test sediments should not be sieved prior to testing unless there is potential concern of similar species, competitors, or predators. Native sediments should always be sieved to remove amphipods from the material to be used as the Control treatment. A 0.5 mm sieve is sufficient to remove the amphipods and sediments should only be dry sieved (manually pushed through the sieve) using only the water present in the sample. These procedures can be performed prior to test set-up and stored under the conditions described above.

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**Title: 10-Day Acute Sediment Toxicity Test with Marine Amphipods (PSEP)**

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## **5.0 EQUIPMENT**

### **5.1 Instrumentation/Equipment**

Microprocessor-controlled recorder, and a digital thermometer

Light meter

DO meter and probe

Salinity meter and probe

pH meter and probe

Ammonia probe meter and ancillary supplies

Microbalance capable of measuring weights to the nearest 0.0001 mg

Environmental test chamber or water bath capable of maintaining test temperature within 1°C

1 L and 250 mL test chambers

Clean filtered seawater

Deionized water

Pipets

Camel hair brushes

Miscellaneous labware (wash bottles, tally counters, culture bowls, etc.)

500 µm stainless steel sieves

Holding cups (food grade plastic is acceptable)

Stir plate and teflon stir bars

Centrifuge for collecting pore water

### **5.2 Apparatus**

#### **5.2.1 Test Area**

The test area consists of a water bath or temperature controlled room with constant monitoring of test temperature and appropriate illumination. The facility will be well ventilated and free of fumes.

#### **5.2.2 Lighting**

Overhead lighting will be ambient and continuous (24-hour).

#### **5.2.3 Test Chambers**

I-L glass jars with a 10-cm internal diameter, covered with a petri dish.

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**Title: 10-Day Acute Sediment Toxicity Test with Marine Amphipods (PSEP)**

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## **6.0 PROCEDURE**

### **6.1 Preparation**

#### **6.1.1 Labware Preparation**

Labware is described as any plastic or glass material used in the laboratory that will come into contact with any of the test substances or organisms in this evaluation. Labware must be cleaned prior to use. Labware will first be soaked in tap or deionized water then scrubbed with a brush on all surfaces using non-phosphate detergent in. Alconox® is a widely used established brand of detergent used in laboratory applications. The clean materials will then be rinsed three times with running deionized water. Labware will then be allowed to soak in a 10% hydrochloric acid bath and afterwards rinsed three times with deionized water. Glass labware will also receive a solvent rinse with reagent grade acetone, and finally rinsed three times with deionized water. Some plastic labware is not resistant to solvents and may be damaged by acetone. Plastic labware such as Teflon can receive a solvent rinse, but all other plastics should be investigated prior to solvent rinsing.

#### **6.1.2 Dilution Water Preparation**

Natural seawater will be obtained from North Hood Canal, sand filtered, and filtered to 0.45µm. Seawater will be adjusted as necessary to maintain a target test salinity. Salinity should be lowered with the addition of high purity deionized water or increased with the addition of bioassay grade sea salts or brine.

#### **6.1.3 Test Organism Acclimation**

For acclimation, amphipods will be held in control sediment with salinity adjusted dilution water. Gentle aeration will be provided for the duration of the acclimation period. Two to three days are sufficient for acclimation to the test conditions. Organisms may be fed a slurry of ground alfalfa or Tetramin™ if held for an extended period.

Amphipods in holding containers will be checked daily before the initiation of a test. Individuals that emerge from the sediment and appear dead or unhealthy will be discarded. If greater than 10% of the amphipods die or appear unhealthy during 48 hours preceding the test, the health of the batch of organisms should be evaluated for use in the proposed testing. This may include an additional day of holding to determine if mortalities or abnormal behavior are due to shipping or acclimation stress, and not indicative of an overly sensitive population.

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## 6.2 Primary Task

### 6.2.1 Pre-Test Analyses

Prior to test initiation, and preferably as soon as sediments are received at the testing facility, pore water should be collected from a homogenized sample from each sediment treatment (including reference and controls). This sample should be analyzed for interstitial salinity, ammonia, and sulfides. The parameters listed in Table 3 are recommendations based upon the tolerance of each species. If conditions within the sediment are outside the tolerance ranges, the project manager and/or client should be notified and possible corrective actions discussed. The most common corrective action involves test chamber overlying water renewal or purging to bring test conditions with tolerance ranges. These procedures are described further in Section 6.2.3.

**Table 4. Species Specific Test Condition Summaries**

Species	<i>Ampelisca abdita</i>	<i>Rhepoxynius abronius</i>	<i>Eohaustorius estuarius</i>	<i>Leptocheirus plumulosus</i> <sup>2</sup>
Total Ammonia (mg/L, pH 7.7)	< 30	< 30	< 60	< 60
Un-ionized Ammonia (mg/L, pH 7.7)	< 0.4	< 0.4	< 0.8	< 0.8
Sulfides	N/A <sup>1</sup>	N/A <sup>1</sup>	N/A <sup>1</sup>	N/A <sup>1</sup>

<sup>1</sup> Specific guidance for sulfide sensitivities have not been well established.

<sup>2</sup> Direct guidance for *L. plumulosus* is not given under PSEP guidelines; however, test conditions are similar to that of *E. estuarius* and described in other guidance documents (EPA 1991, 1994).

### 6.2.2 Test Sediment Addition

Test sediment will be prepared using glassware cleaned according to Section 6.1.1, pre-cleaned glassware of a disposable nature, or non-toxic food grade plastic. All test chambers should be labeled accordingly with corresponding random number positions. After setup, the test chambers are distributed throughout the testing area based upon their position numbers. All 5 treatment replicates, including the corresponding Water Quality Surrogate (see Section 6.2.3), should be included in the randomized test matrix.

If necessary, sieving of the control sediment and/or test treatments will be performed (see Section 4.0). On the day before the test begins, each test sediment sample will be thoroughly homogenized within its storage container, and an aliquot added to a test chamber depth of 2 cm.

The sediment within the test chamber will be settled by tapping the test chamber against the side of the hand. Prepared seawater is gently added up to the 950-mL level (about 775 mL). A solid disk attached to a rod is placed inside the chamber to limit the suspension of the sediment into the water column by diffusing the water down the inside of the test chamber. The disc should be maintained just above the water surface as the

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test chamber is filled. The sample is left overnight with gentle aeration to allow suspended particles to settle and equilibrium to be established between sediment and overlying water before the amphipods are added.

### 6.2.3 Sample Adjustments

If the water quality conditions in the test chamber are not suitable to support the selected amphipod species, it may be necessary to adjust those conditions to within tolerance limits. The two most common parameters which may require attention include interstitial salinity and ammonia. Water quality conditions (exclusive of contaminants) should be within the tolerance limits of the test species to remove the impact of their interference on the determination of toxic effects. Depending upon the program, manipulations to the test treatments may be performed to correct any deviations. Unfortunately, these manipulations may also alter the level of contaminants through purging or alter their available chemical state (salinity or pH change). Best professional judgment must be employed when deciding to manipulate the sample treatments and should always involve discussion with the client or regulatory agency. If manipulations are performed to the test treatments, the associated Control and Reference sediment should be treated in the same manner.

Generally, adjustments to the interstitial salinity of the sediments are not desirable. Exceptions to this may be sediments with very low interstitial salinities that are destined for open ocean disposal (~32-35 ppt). Salinity may be adjusted by replacing the overlying water within the test chambers with water of salinity equal to, or slightly greater than (or slightly less than if lowering), the target test salinity. The test chamber water should be removed through siphoning or pumping the water out to a level just above the test sediment. Care should be taken not to remove any sediment during this process. Prepared seawater is gently added up to the 950-mL level. A solid disk attached to a rod is placed inside the chamber to limit the suspension of the sediment into the water column by diffusing the water down the inside of the test chamber. The disc should be maintained just above the water surface as the test chamber is filled.

For sediments with pore water ammonia concentrations exceeding those values listed in Table 4, purging may be required to bring the test chambers conditions within acceptable limits. In most cases this should be determined in the pre-test pore water analyses (Section 6.2.1). General procedures for purging of the test chambers are described in further detail in the SMARM clarification paper “Ammonia and Amphipod Toxicity Testing” (SMARM clarification paper: 06/15/02). Additional sacrificial surrogate chambers should be created to monitor pore water ammonia levels during the acclimation process. Overlying water exchanges are conducted in the same manner as the overlying water renewal for salinity adjustment described above. Purging should be conducted twice daily until the pore water ammonia concentrations are below the threshold values. Pore water ammonia levels should be monitored every 1-3 days during the purging process. Overlying ammonia levels should also be measured as part of the monitoring procedure

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as it gives an estimate of ammonia reduction without the breakdown of a surrogate chamber. Once the pore water ammonia has been reduced below the threshold values, purging should be terminated and the testing period can commence. Depending upon the program, purging may or may not be continued after test initiation. It may be possible in highly biogenic sediment that ammonia may increase again over the course of the test if renewals are discontinued.

#### 6.2.4 Reference Toxicity Test

During this 96-hour toxicity test with marine amphipods and a test substance, five concentrations of a reference substance (ammonium chloride) with 10 test organisms will be used to assess the health of the test organisms. Three test chambers per reference concentration may be used. One concentration will be the 96-hour LC<sub>50</sub>. The other four concentrations will be selected to bracket the LC<sub>50</sub>. The LC<sub>50</sub> values will be compared with historical data from definitive bioassays with the reference substance. The results of the 96-hour mortality, determined during this study, will be reported and used in combination with control mortality to characterize the health of the test organisms. Table 5 summarizes the test conditions for conducting a 96-hour water-only reference toxicant test.

**Table 5. Conditions for Performing 4-Day Water-Only Reference Testing on Marine Amphipods**

Test type	Static Non-renewal
Test duration	4 Day
Lighting	Dark and Constant <sup>1</sup>
Test chamber size	250-mL glass beaker (minimum)
Test solution volume	200-mL (minimum)
Renewal of test solution	None
No. of organisms per chamber	10 recommended (minimum of 5)
No. of replicates per treatment	3
Feeding	None
Test solution aeration	None unless needed to maintain DO levels above 60% saturation

<sup>1</sup> In the absence of sediment, amphipods will continue to attempt to bury into the bottom of the chamber. Keeping the amphipods in the dark will lessen this digging behavior thus reducing undue stress on the test organisms.

The results of the ammonia reference-toxicant may be compared to the ammonia concentrations observed within the test samples to assist in correlating any ammonia related effects within a specific batch of organisms. Table 6 summarizes the published threshold ammonia concentrations for each species.

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**Table 6. Threshold sediment interstitial ammonia levels for triggering ammonia reference toxicant tests.**

Interstitial Ammonia (mg/L @ pH 7.7)	<i>Ampelisca abdita</i>	<i>Rhepoxinius abronius</i>	<i>Eohaustorius estuarius</i>	<i>Leptocheirus plumulosus</i> <sup>1</sup>
Total	>15	>15	>30	>30
Unionized	>0.2	>0.2	>0.4	>0.2

<sup>1</sup> Direct guidance for *L. plumulosus* is not given under PSEP guidelines; however, test conditions are similar to that of *E. estuarius* and described in other guidance documents (EPA 1991, 1994).

### 6.2.3 Reference and Control Sediment

During this 10-day toxicity test with marine amphipods on project sediment(s), reference sediment(s) will be used to provide a site-specific basis for comparison of potentially toxic and non-toxic conditions. Control sediment, collected during amphipod collection at the same site, will be used to determine the condition of the amphipods.

### 6.2.5 Water Quality

During routine test observations, a daily record of test room or water bath temperatures and test chamber aeration should be made.

In order to limit the impact of disturbance on the test organisms, all water quality measurements during the testing procedure will be performed in a surrogate water quality only chamber. In addition to the five test treatment replicates, a minimum of three additional surrogate chambers should also be tested; one for use as a water quality surrogate (WQS), and two to be utilized at test initiation and termination for pore water analyses. Surrogate chambers should be treated in the same manner as the test replicates. This includes randomization among the test treatments and addition of test animals. Additional pre-test surrogate chambers may also be required to monitor pore water salinity, ammonia, or sulfide manipulations.

After one day of acclimation after sediment and overlying water layering (test day 0), an initial set of water quality parameters will be measured in the overlying water of the WQS for each test treatment. The water quality parameters include temperature, dissolved oxygen (DO), pH, salinity, total ammonia, and total sulfides. In addition, a surrogate replicate from each test treatment will be sacrificed in order to extract pore water via centrifugation for subsequent analysis of ammonia and sulfides. Prior to test initiation, these initial water quality measurements must be reviewed to ensure that they are within the testing parameters. Test initiation should be postponed until any deviations are addressed and corrected.

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On test days 1 through 9, temperature, DO, pH, and salinity will be measured in the water quality surrogate chamber of each treatment. At test termination (test day 10) the full suite of measurements will be repeated as on day 0.

### **6.2.6 Test Organism Addition**

Amphipods are sieved from the holding sediment (500 µm sieve) and transferred to a sorting tray containing water of the holding temperature and salinity. Active, healthy amphipods are randomly selected from the sorting tray and sequentially distributed among dishes containing approximately 15 mL of dilution seawater until each dish contains 5 individuals. Prior to addition to the test chambers, the number of organisms is verified by recounting the individuals within the dish as well as confirming health and appearance. Unacceptable amphipods are discarded and replaced prior to introduction.

Twenty animals (4 dishes of 5 animals each) are then added to the randomly positioned test chambers. Addition should occur with minimal disruption of the sediment by gently pouring the water and amphipods from the sorting dishes into the test chamber. Any amphipods remaining in the dish should be gently washed into the test chamber. After addition, the test chamber is marked to confirm organism addition, recovered, and aeration restored. Any amphipods that do not bury within 15 minutes will be removed and replaced (*Ampelisca abdita* should be allowed one hour for burial).

### **6.2.5 Test Initiation**

The test is initiated when the test organisms are distributed to each test chamber.

### **6.2.6 Test Observations**

Notes are made on sediment appearance and unusual conditions. This can include fungal and algal growth. The number of amphipods that have emerged from the sediment, either floating on the water surface or lying on top of the sediment is recorded. Amphipods that are floating on the surface can be released from the surface tension by dropping a small drop of water (from the test chamber) with a pipette. Care must be taken not to cross-contaminate beakers. Dead animals either on the water or sediment surface are not removed during the exposure period. A list of observation types and their corresponding codes are detailed in Table 7.

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**Table 7. Observation Key for Recording Test Observations**

Normal	N
No Burrows	NB
Body on surface (mortality). Can indicate a corpse or a molt.	M
Emergence (actively swimming in water column, or walking on sediment surface; not burrowing)	E
Growth. Indicative of fungal, algal, or bacterial mats	G
No Air Flow	D
Floating on surface. Animals caught in surface tension of water.	FOS
Water too cloudy/turbid for observation	TC

### 6.3 Post-task

The bioassay is terminated on day 10. After final observations are performed, the contents of each test chamber are sieved through a 0.5-mm sieve. A gentle spray of seawater is used to wash the sediment through the sieve. Material retained on the sieve is transferred to a clean sorting vessel containing seawater of a similar salinity and temperature as the test. The numbers of live and dead amphipods are recorded. An amphipod is considered alive if there is any sign of movement (e.g., pleopod twitching or response to gentle prodding). Recoveries may not equal 20 due to the decomposition of dead animals through the test. Although not commonly conducted, there is also a procedure for evaluating the ability of the amphipods (excluding *A. abdita*) to rebury into Control sediment. This sublethal endpoint is discussed in further detail in PSEP 1995.

#### Results Needed:

- Percent mortality for each treatment
- Mean water quality values by treatment
- LC<sub>50</sub> and 95% confidence limits (for ref. tox.)
- Reburial

In screening tests, the responses of amphipods in collected test sediments are compared to control and reference site sediments.

### 6.4 Reporting

The report may include, but will not be limited to, the following:

- Name and address of the laboratory conducting the study, and dates on which the study was initiated and completed.
  - The name of the Study Director, other scientists or professionals, and supervisory personnel involved in the study.
  - Objectives as stated in the protocol.
  - A description of the methods used.
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- Transformations, calculations, or operations performed on the data, a summary and analysis of the data, and a statement of the conclusion drawn from the analysis.
- The test substance identified by code number and the date each sample was used.
- The number of organisms used in the study.
- Concentrations of exposure and exposure method.
- Any circumstances that may have affected the quality or integrity of the data, including deviations from test protocols or Standard Operating Procedures.
- The location where raw data and the final report will be stored.
- Additions or corrections to a final report will be in the form of an amendment by the Study Director. The amendment will clearly identify that part of the final report that is being altered and the reason(s) for the alteration(s). The amendment will be signed and dated by the Study Director.

The master copy of the final report will be signed and dated by the Study Director.

## 7.0 HEALTH AND SAFETY CONSIDERATIONS

Proper laboratory protection, including lab hood or ventilation system, lab coat, closed-toe shoes, gloves and safety glasses, is required when working with chemicals and unprocessed samples.

Refer to the Port Gamble Laboratory's Chemical Hygiene Plan and Health and Safety Plan at [S:\Health and Safety](#) for procedures to ensure safe operation in the laboratory and for contingency plans in the event of an accident or emergency.

For specific chemical health and safety information, refer to the Safety Data Sheet log.

## 8.0 PERSONNEL

Any laboratory personnel demonstrating competence with this method may perform the procedure.

## 9.0 QUALITY ASSURANCE REQUIREMENTS (ACCEPTANCE CRITERIA)

This study will be conducted according to the Standard Operating Procedures of the Port Gamble Laboratory which are in effect during the time the study is being performed. In the case where there is a conflict between the other SOPs and this protocol, the protocol will be the definitive procedure.

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Usually tests would be unacceptable if the following conditions occurred:

- More than 10% of the organisms die in the Control treatment.

Test data will need to be evaluated and qualified if:

- All test chambers were not identical.
- Treatments were not randomly assigned to test chambers.
- Test organisms were not randomly or impartially distributed to test chambers.
- All test animals were not from the same population, were not all of the same species, or were not of acceptable quality.
- Reference sediment and controls were not included in the test.
- Amphipods were maintained in the laboratory for less than two days or greater than ten days, unless the effect of prolonged maintenance in the laboratory has been shown to have no significant effect on sensitivity.
- Temperature, DO, pH, salinity, and ammonia were not measured, or were not within acceptable range.
- Test organisms were not acclimated at the test temperature and salinity at least 24 hours before they were placed in test chambers.
- Aeration to the test chamber was off for an extended time such that the DO levels dropped below acceptable limits and was associated with mortality.
- Response criteria were not monitored in a blind fashion.

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## 10.0 REFERENCE DOCUMENTS

Puget Sound Water Quality Authority. Revised July 1995. "Recommended Guidelines for Conducting Laboratory Bioassays on Puget Sound Sediments." Prepared for U.S. EPA Region 10, Office of Puget Sound. Seattle, W A.

Sediment Management Annual Review Meeting Clarification Paper. Barton, J. "Ammonia and Amphipod Toxicity Testing." June 15, 2002.

Sediment Management Annual Review Meeting Clarification Paper. Kendall, D.R. "Clarification on the Use of the Amphipod, *Eohaustorius estuarius*, Relative to Grain Size and Salinity." October 20, 1999.

Sediment Management Annual Review Meeting Clarification Paper. Warner, L.C. "Reporting Ammonia LC<sub>50</sub> Data for Larval and Amphipod Bioassays". October 3, 2001.

Sediment Management Annual Review Meeting Clarification Paper. Michelson, T. "Statistical Evaluation of Bioassay Results". July 25, 1996.

ASTM E 1367. "Standard Test Method for Measuring the Toxicity of Sediment Associated Contaminants with Estuarine and Marine Invertebrates." Annual Book of Standards. Volume 11.06 "Biological Effects and Environmental Fate; Biotechnology." American Society of Testing and Materials, 100 Barr Harbor Drive, West Conshohocken, PA. 2006.

Norberg-King, T.J. 1998. An interpolation estimate for chronic toxicity: The Icp approach. Technical Report 05-88, National Effluent Toxicity Assessment Center, Environmental Research Laboratory, U.S. Environmental Protection Agency, Duluth, Minnesota.

USACE/USEPA (U.S. Army Corps of Engineers/U.S. Environmental Protection Agency). 1991. Evaluation of Dredged Material Proposed for Ocean Disposal - Testing Manual." Office of Water, Washington, DC. EPA/503/8-91/001. February, 1991.

USACE/USEPA. 1994. "Methods for Assessing the Toxicity of Sediment-associated Contaminants with Estuarine and Marine Amphipods." Office of Research and Development, Washington, DC. EPA/600/R-94/025.

USACE/USEPA. 1998. "Evaluation of Dredged Material Proposed for Discharge in Waters of the U.S. - Testing Manual." Office of Water, Washington, DC. EPA/823/B-98/004. February, 1998.

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## **11.0 APPENDIX OF CHANGE**

- 08/20/15 Changed test organism acclimation section to reflect a 10% mortality threshold in assessing the organism's health
- 05/23/16 Added "uncontrolled" statement to SOP and updated Health and Safety section
- 05/09/17 Updated health and safety information, removed branding, added review documentation section. Added "proprietary information" statement to footer.

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**Title: Chronic Larval Sediment Toxicity Test (PSEP Guidelines)**


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## 1.0 SCOPE

To evaluate the chronic toxicity of marine sediments Sediment testing will be conducted as defined in **Dinnel and Stober (1995)**, **Standard Methods (APHA 1985)**, **ASTM (1989)**, and **Recommended Guidelines for Conducting Laboratory Bioassays on Puget Sound Sediments (1995)**.

## 2.0 SUMMARY OF TEST

### 2.1 Approach

Number of samples	≥ 1
Number of replicates per test sediment	5
Number of controls	1
Number of replicates per control	5
Number of reference sediments	1
Number of replicates per reference sediment	5
Test chambers	1 L glass beaker or comparable wide mouth glass jar (10 cm internal diameter)
Test sediment volume	18 g of sediment per container
Overlying water	900 mL of 28‰ salinity, clean, filtered, seawater with a maximum holding time of 2 days
Renewal of overlying water	None
Number of test organisms per chamber	20,000-40,000 embryos for Bivalves, 25,000 embryos for Echinoderms
Type of biological observations	Survival, Development
Times of biological observations	Post test
Type of physical observations	Room or bath temperature continuous, light daily
Types of water quality analyses	DO, temperature, salinity, pH; ammonia and sulfides (Program Dependent)
Times of water quality samples	DO, temperature, salinity, and pH measured in a surrogate test chamber daily. Ammonia and total sulfide samples taken at the beginning and end of the test.
Aeration	Gentle aeration is applied to all chambers if Dissolved Oxygen levels fall below 6.0 mg/L.
Sediment holding time	Samples must be stored in the dark at $4 \pm 2^{\circ} \text{C}$ with no headspace or headspace filled with nitrogen gas.

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**Title: Chronic Larval Sediment Toxicity Test (PSEP Guidelines)**


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## 2.2 Physical Requirements

Species	<i>Dendraster excentricus</i>	<i>Strongylocentrotus purpuratus</i>	<i>Mytilus galloprovincialis</i>	<i>Crassostrea gigas</i>
DO (mg/L)*	> 4.8	> 4.8	> 4.8	> 4.6
Temperature (°C)	15 ± 1	15 ± 1	16 ± 1	20 ± 1
Salinity (ppt)	28 ± 1	28 ± 1	28 ± 1	28 ± 1
pH	Ambient	Ambient	Ambient	Ambient
Lighting	14 hours light : 10 hours dark at 50-100 foot-candles	14 hours light : 10 hours dark at 50-100 foot-candles	14 hours light : 10 hours dark at 50-100 foot-candles	14 hours light : 10 hours dark at 50-100 foot-candles
Ammonia	< 0.14 mg/L unionized	< 0.14 mg/L unionized	< 0.13 mg/L unionized	< 0.13 mg/L unionized
Aeration	Gentle, if D.O. falls below 6.0 mg/L	Gentle, if D.O. falls below 6.0 mg/L	Gentle, if D.O. falls below 6.0 mg/L	Gentle, if D.O. falls below 6.0 mg/L

\*60 percent saturation at 15°C and 28 ppt.

## 2.3 Biological Requirements

Feeding None

Life stage Larval Stage used within 2 hours of fertilization depending on test species.

## 3.0 TEST ORGANISM

The test organism should be selected based on availability, sensitivity to test materials, tolerance to ecological conditions, ecological importance, and ease of handling in the laboratory. Ideally, organisms with wide geographical distribution should be selected so test results can be compared among laboratories with similar organisms. The organisms for this protocol are *D. excentricus*, *S. purpuratus*, *M. galloprovincialis*, and *C. gigas*.

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### 3.1 Test Organism Specifications

Species:	<i>Dendraster excentricus</i>	<i>Strongylocentrotus purpuratus</i>	<i>Mytilus galloprovincialis</i>	<i>Crassostrea gigas</i>
Age:	Larval Stage used within 2 hours of fertilization	Larval Stage used within 2 hours of fertilization	Larval Stage used within 2 hours of fertilization	Larval Stage used within 2 hours of fertilization
Source:	In-house collection; Aquatic Toxicology Support, Bremerton, WA; Dave Gutoff, San Diego, CA	Dave Gutoff, San Diego, CA; In-house collection; Aquatic Toxicology Support, Bremerton, WA	Taylor Shellfish, Shelton, WA; Aquatic Research Organisms, Hampton, NH	In-house collection; Taylor Shellfish, Shelton, WA; Aquatic Toxicology Support, Bremerton, WA

### 3.2 Test Organism Care

Records of the stock shipments will be kept, including original source, feeding regime, and holding water characteristics

## 4.0 TEST SUBSTANCE

The test substance will be labeled, properly stored, and tracked by internal chain-of-custody procedures throughout its tenure at the Port Gamble Laboratory. The test substance will not be heated, filtered, distilled, frozen, or otherwise altered without prior written consent by the Client.

The test substance is stored at 0 - 6°C in a secure and distinct storage area.

## 5.0 EQUIPMENT

### 5.1 Instrumentation/Equipment

Microprocessor-controlled recorder, and a digital thermometer

Light meter

DO meter and probe

Salinity meter and probe

pH meter and probe

Ammonia probe meter and ancillary supplies

Method of measuring total sulfides

1 L test chambers

Clean filtered seawater

Pipets

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Miscellaneous labware (wash bottles, tally counters, culture bowls, etc.)  
Centrifuge for collecting pore water  
20 mL Scintillation or 25 mL shell vials  
Syringe (to inject KCl into echinoderms) with 18-22 gauge needle  
Pasteur pipets and bulbs  
Ice bath or refrigerator  
Compound microscope  
Neubauer hemocytometer  
Sedgwick-Rafter (or equivalent) counting cell (1 mL)  
Small siphon hose (2ft. long, 3/16 - 1/4 in diameter)  
Laboratory timer  
Controlled temperature water bath or room  
100-mL graduated cylinder  
Perforated plunger

## **5.2 Reagents**

0.5 M KCl  
5% Buffered formalin  
Copper sulfate  
10% hydrochloric acid  
Acetone  
5% buffered formalin

## **5.3 Apparatus**

### **5.3.1 Test Area**

The test area consists of a room with constant temperature and appropriate illumination. The facility will be well ventilated and free of fumes.

### **5.3.2 Lighting**

Continuous overhead lighting will be at 50-100 foot-candles (550-1050 Lux).

### **5.3.3 Test Chambers**

I-L glass chambers  
20 mL glass scintillation or 25 mL glass shell vials

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## **6.0 PROCEDURE**

Prior to use, all glassware and plasticware will be thoroughly cleaned.

### **6.1 Preparation**

#### **6.1.1 Glassware Preparation**

Glassware will first be soaked in deionized water then scrubbed with a brush on all surfaces using non-phosphate detergent in deionized water. Glassware will be rinsed three times with running deionized water. Glassware will then be rinsed in 10% hydrochloric acid, rinsed three times with deionized water, rinsed once with reagent grade acetone, and finally rinsed three times with deionized water.

#### **6.1.2 Dilution Water Preparation**

Filtered seawater collected from North Hood Canal will be diluted to  $28 \pm 1$  ppt salinity using deionized water. Seawater will be held for a maximum of 2 days.

#### **6.1.3 Test Organism Acclimation**

Stock cultures will be acclimated in the same dilution water and at the same temperature as in the test procedures. Short-term culture logs will be maintained throughout the holding period.

## **6.2 Primary Task**

### **6.2.1 Test Sediment Addition**

Test sediment will be prepared using glassware cleaned according to Section 6.1.1, pre-cleaned glassware of a disposable nature, or non-toxic food grade plastic.

Eighteen grams of reference or test sediment is added to each chamber. 900 mL of filtered seawater (28 ppt salinity) is added to each test chamber. Two control series are prepared consisting of clean seawater without sediment. One series is used as a duplicate control in order to monitor embryo development. The sediments are suspended by vigorously shaking for 10 seconds. Test chambers will be randomized, and the mixture will be allowed to settle for four hours prior to embryo induction.

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### 6.2.2 Reference Toxicant Test

Concurrent with this toxicity test, five concentrations of a reference substance are used to assess the health of the test organisms. Three replicates per reference concentration will be used. Five concentrations will be selected to bracket the 48-hour LC<sub>50</sub>. The LC<sub>50</sub> values will be compared with historical data from definitive bioassays. The results of this survival and development test, conducted during this study, will be reported and used in combination with control survival and development to characterize the health of the test organisms.

### 6.2.3 Test Organism Spawning and Addition

To collect gametes for testing with bivalve species, the adult organisms are placed in clean seawater and acclimated to the target test salinity (test dependant) and temperature (16°C for *Mytilus* and 20°C for *Crassostrea*) for approximately 20 minutes. The water bath temperature is then increased 5°C over a period of 15 minutes. Bivalves are maintained at this elevated temperature and monitored for spawning individuals. Spawning animals are removed from the water bath and placed in individual containers with seawater. Gametes from at least two males and two females are used to initiate the test. Once sufficient eggs and sperm had been collected, the eggs are screened through 60-µm mesh to remove any detritus or feces and a homogenized sperm solution added to the egg solutions. Egg-sperm solutions are periodically homogenized with a perforated plunger during the fertilization process. Approximately one hour after fertilization, embryo solutions are checked for fertilization and cell division. Only those embryo stocks with >90% cell division are used to initiate the tests. Density of the embryo stock solution is determined by counting the number of embryos in a subsample of stock solution. For bivalve species, approximately 20,000 - 40,000 embryos will be added to test chambers within 2 hours of fertilization.

To collect gametes required for testing echinoderm species, spawning is induced in the adult organisms by the injection of 0.5 to 1.0 mL of 0.5 M KCl into the coelomic cavity through the perisotomal membrane. The injection is performed while the adult animal is out of water. Females will release orange (*S. purpuratus*) or purple (*D. excentricus*) eggs and males of both species will release cream-colored sperm. Once release has been initiated, each adult is inverted over a 50 to 100 mL beaker with filtered seawater at 15 degrees Celsius and gametes are allowed to accumulate for approximately 15 minutes. Once sufficient eggs and sperm had been collected, the eggs are transferred to a larger beaker with cold filtered seawater and a homogenized sperm solution (taken from several males) is added to the egg solutions. Egg-sperm solutions are periodically homogenized with a perforated plunger during the fertilization process. Approximately one half-hour after fertilization, embryo solutions are checked for fertilization. Only those embryo stocks with >90% fertilization are used to initiate the tests. Density of the embryo stock solution is determined by counting the number of embryos in a subsample of stock solution.

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**Title: Chronic Larval Sediment Toxicity Test (PSEP Guidelines)**

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Approximately 25,000 embryos will be added to test chambers within 2 hours of fertilization.

The test is initiated by randomly allocating an aliquot of the embryo stock solution into each test chamber four hours after sediments are shaken and within two hours of egg fertilization. Embryos are held in suspension during initiation using a perforated plunger. The test chambers are covered and incubated for 48 hours or longer under the conditions specified in Section 2.2.

For embryos/larvae test, a perforated plunger is used to mix the embryos/larvae at the test initiation. Approximately 25,000 echinoderm or 20,000-30,000 bivalve embryos will be added to test chambers within 2 hours of fertilization.

In order to determine the initial embryo concentration, five 10-mL samples should be collected from the control culture and preserved using 1-mL 5% buffered formalin.

#### **6.2.4 Test Initiation**

The test is initiated when the first organism enters a test chamber.

#### **6.2.5 Test Substance Renewal**

No test substance renewals are required by this protocol.

#### **6.2.6 Test Measurements**

**Water Quality.** All probes will be cleaned thoroughly before initial use. Data collection will be performed on each samples respective surrogate chamber and recorded. The probes will be rinsed with de-ionized water between each sample. DO, temperature, salinity, and pH will be measured from the overlying water in the surrogate chamber daily. Ammonia and total sulfides should be measured in the overlaying water at least at the beginning of the test.

**Biological.** Larvae will be scored for normal development according to ASTM guidelines.

#### **6.2.7 Test Termination**

For echinoderm species, the test is terminated at 48 hours or when greater than 90% of the embryos in the duplicate seawater control have reached normal development (whichever is later and within 96 hours). For bivalve species, the test is terminated at 48 hours or when greater than 95% of the embryos in the duplicate seawater control have reached normal development (whichever is later and within 60 hours).

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The water and the larvae overlying the settled sediment in each container are carefully mixed in order to suspend larvae and prevent disturbance of the sediment. The overlying water and larvae are then placed into a clean, I-L beaker. The water is then mixed, and three 10-mL aliquots of the sample are removed and placed into 20-mL scintillation vials or shell vials. The contents of each vial are preserved with 1.0-mL of 5% buffered formalin, and the caps are securely replaced on the vials.

Preserved samples are examined on Sedgwick-Rafter cells (if using scintillation vials) or in the shell vials. Normal and abnormal larvae are counted to determine the percent normal development. In addition, percent survival is determined using the appropriate method outlined in **Recommended Guidelines for Conducting Laboratory Bioassays on Puget Sound Sediments (1995)**.

### 6.3 Post-task

- PSEP 1995 Recommendations:

Calculate the percent mortality for each replicate:

$$\text{Mortality} = 100 \times (1 - (\text{No. of surviving test larvae} / \text{No. of control larvae}))$$

Calculate the percent abnormality for each replicate:

$$\text{Abnormality} = 100 \times (1 - (\text{No. of abnormal larvae} / \text{No. of normal and abnormal survivors}))$$

Calculate the combined larval mortality/abnormality:

$$\text{Combined larval mortality/abnormality} = 100 \times (1 - (\text{No. of surviving normal larvae} / \text{No. of embryos inoculated}))$$

- Conventional endpoint calculations typically reported for PSEP testing programs:

Endpoint Calculation	Sample Type	
	Control	Reference and Project
Proportion Normal	No. of surviving normal larvae / No. of normal and abnormal survivors	
Proportion Survival	No. of normal and abnormal survivors / No. of embryos inoculated	
Normal Survivorship (Combined Proportion Normal)	No. of surviving normal larvae / No. of embryos inoculated	No. of surviving normal larvae / Mean No. normal in the Control

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**Results Needed:**

- Individual replicate, mean, and standard deviation values for percent survival/mortality, percent normality/abnormality, and combined larval mortality/abnormality
- Plot of dose-response curve at test termination (48 or 72 hours)
- LC<sub>50</sub> value for mortality, EC<sub>50</sub> value for abnormality
- 95% confidence limits of LC<sub>50</sub> value and EC<sub>50</sub> value
- Tables showing biological, chemical, and physical data

**6.3.1 Method**

LC<sub>50</sub> and EC<sub>50</sub> values and 95% confidence limits will be determined using a computer approach published by Norberg-King (1988) of the U.S. EPA and a commercial statistic software program. The Inhibition Concentration percentage, or ICp, approach to calculating point estimates of toxicity (i.e., LC<sub>50</sub> and EC<sub>50</sub>) is based upon a monotonic smoothing technique of biological response versus concentration. Bootstrapped estimates of mean response at each concentration allow for distribution-free estimates of standard error and confidence intervals. The result is a nonparametric statistical test that requires no assumptions of normality or homogeneous variance and is robust enough to accommodate a wide variety of biological responses.

**6.4 Reporting**

The report may include, but will not be limited to, the following:

- Name and address of the laboratory conducting the study, and dates on which the study was initiated and completed.
  - The name of the Study Director, other scientists or professionals, and supervisory personnel involved in the study.
  - Objectives as stated in the protocol.
  - A description of the methods used.
  - Information regarding organisms used.
  - All water quality measurements.
  - Transformations, calculations, or operations performed on the data, a summary and analysis of the data, and a statement of the conclusion drawn from the analysis.
  - The test substance identified by code number and the date each sample was used.
  - The number of organisms used in the study.
  - Concentrations of exposure and exposure method.
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- Any circumstances that may have affected the quality or integrity of the data, including deviations from test protocols or Standard Operating Procedures.
- The location where raw data and the final report will be stored.
- Additions or corrections to a final report will be in the form of an amendment by the Study Director. The amendment will clearly identify that part of the final report that is being altered and the reason(s) for the alteration(s). The amendment will be signed and dated by the Study Director.

The master copy of the final report will be signed and dated by the Study Director.

## 7.0 HEALTH AND SAFETY CONSIDERATIONS

Proper laboratory protection, including lab hood or ventilation system, lab coat, closed-toe shoes, gloves and safety glasses, is required when working with chemicals and unprocessed samples.

Refer to the Port Gamble Laboratory's Health and Safety Plan at [S:\Health and Safety](#) for procedures to ensure safe operation in the laboratory and for contingency plans in the event of an accident or emergency.

For specific chemical health and safety information, refer to the Safety Data Sheet log.

## 8.0 PERSONNEL

Any laboratory personnel demonstrating competence with this method may perform the procedure.

## 9.0 QUALITY ASSURANCE REQUIREMENTS (ACCEPTANCE CRITERIA)

This study will be conducted according to the Standard Operating Procedures which are in effect during the time the study is being performed. In the case where there is a conflict between the other SOPs and this protocol, the protocol will be the definitive procedure.

Test acceptability criteria are:

- $\geq 90\%$  survival of embryos introduced into control test chambers.
- $\geq 70\%$  of embryos demonstrate normal development in the control.

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## 10.0 REFERENCE DOCUMENTS

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Chapman, G.A., et al. 1995. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms, 1st edition. U.S. EPA Environmental Monitoring Systems Laboratory, Cincinnati, Ohio, EPA/600/R-95/136.

Sediment Management Annual Review Meeting Clarification Paper. Warner, L.C. "Reporting Ammonia LC<sub>50</sub> Data for Larval and Amphipod Bioassays". October 3, 2001.

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Norberg-King, T.J. 1988. An interpolation estimate for chronic toxicity: The ICp approach. Technical Report 05-88, National Effluent Toxicity Assessment Center, Environmental Research Laboratory, U.S. Environmental Protection Agency, Duluth, Minnesota.

USACE/USEPA (U.S. Army Corps of Engineers/U.S. Environmental Protection Agency). 1991. Evaluation of Dredged Material Proposed for Ocean Disposal - Testing Manual." Office of Water, Washington, DC. EPA/503/8-911001. February, 1991.

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PTI Environmental Services. 1991. Draft Recommended Guidelines for Conducting Laboratory Bioassays on Puget Sound Sediments. Prepared for U.S. EPA, PTI Contract C744-12.

Puget Sound Water Quality Authority. Revised July 1995. Recommended Guideline for Conducting Laboratory Bioassays on Puget Sound Sediments. Prepared for U.S. EPA Region 10, Office of Puget Sound, Seattle, WA.

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## 11.0 APPENDIX OF CHANGE

- 04/28/16 Updated logo
- 05/26/16 Added “uncontrolled” statement to SOP and updated Health and Safety section
- 05/09/17 Updated health and safety information, removed branding, added review documentation section. Added “proprietary information” statement to footer. Updated organism suppliers. Added the use of shell vials as an alternative to scintillation vials. Removed reference toxicant and retesting time frames from test acceptability criteria in section 9.0. Added clarification papers from Seattle USACE DMMO. Updated post-task calculation options.

**Title: 20-Day Chronic Growth and Survival Test with  
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## 1.0 SCOPE

To determine the chronic toxicity of marine sediments on the marine polychaete *Neanthes arenaceodentata*. Sediment toxicity testing will be conducted according to guidelines presented in **ASTM E1611, Recommended Guidelines for Conducting Laboratory Bioassays on Puget Sound Sediments (1995)**, and the various updates presented during the Annual Sediment Management Review meetings (SMARM).

## 2.0 SUMMARY OF TEST

**Table 1 Test Condition Summary**

Sample storage conditions	4°C, dark minimal head space
Recommended Sediment Holding Time:	≤8 weeks (56 days)
<b>Test Species</b>	<b><i>Neanthes arenaceodentata</i></b>
Age class	Juvenile (2-3 weeks post-emergence)
<b>Test Procedures</b>	ASTM, PSEP 1995 with SMARM revisions
Regulatory program	SMS, DMMP, or other as mandated by the associated program
Test type/duration	20-Day static renewal
Test chamber	1-Liter glass beaker or jar
Exposure volume	175 mL (2cm) sediment/ 775 mL water
Replicates per treatment	5 + 2 surrogate chambers (one used for WQ measurements throughout the test)
Control / Diluent water	North Hood Canal, sand filtered
Test Lighting	Continuous
Aeration	Continuous from test initiation: 100 bubbles per minute
Test temperature	Recommended: 20 ± 1 °C
Test Salinity	Recommended: 28 ± 2 ppt
Test dissolved oxygen	Recommended: > 4.6 mg/L (60% saturation @ 20°C and 28 ppt salinity) <sup>1</sup>
Test pH	Recommended: 7 – 9 <sup>2</sup>
Organisms/replicate	5
Feeding	40 mg/jar every other day (8mg/ind every other day)
Water renewal	Water renewed every third day (1/3 volume of exposure chamber)

<sup>1</sup> PSEP guidance is not specific on dissolved oxygen limits. The value of 60% saturation is based on ASTM 2006.

<sup>2</sup> pH is monitored as a water quality parameter. There are generally no control limits for pH; however measurements of pH may be useful in interpreting results (Ecology 2003).

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## 2.2 Physical Requirements

DO	>4.6 mg/L (60% Saturation)
Temperature	20 ± 1°C
Salinity	28 ± 2 ppt (PSEP); 28 – 36 ppt (ASTM)
pH	7.0 - 9.0
Lighting	Continuous ambient light at approximately 50-100 foot-candles (550-1050 lux)

## 2.3 Biological Requirements

Feeding	Organisms will be fed ground TetraMin® on an every-other-day basis. The amount of food provided will be approximately 8 mg (dry weight) per juvenile <i>N. arenaceodentata</i>
Life stage	Juvenile worms (2-3 weeks, 0.25-1.0 mg),

## 3.0 TEST ORGANISM

The test organism should be selected based on availability, sensitivity to test materials, tolerance to ecological conditions, ecological importance, and ease of handling in the laboratory. Ideally, organisms with wide geographical distribution should be selected so test results can be compared among laboratories with similar organisms. The organism for this protocol is *Neanthes arenaceodentata*.

### 3.1 Test Organism Specifications

Species:	<i>Neanthes arenaceodentata</i>
Source:	Aquatic Toxicology Support, Bremerton, WA
Age:	Juvenile Worms (2-3 weeks, 0.25-1.0 mg), laboratory cultured

## 4.0 TEST SUBSTANCE

The test substance will be labeled, properly stored, and tracked by internal chain-of-custody procedures throughout its tenure at the Port Gamble Laboratory. The test substance will not be heated, filtered, distilled, frozen, or otherwise altered without prior written consent by the Client. The test substance is stored at 0 - 6°C in a secure and distinct storage area.

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## **5.0 EQUIPMENT**

### **5.1 Instrumentation/Equipment**

Thermometer  
Light meter  
DO meter and probe  
Salinity meter and probe  
pH meter and probe  
Ammonia probe meter and ancillary supplies  
Microbalance capable of measuring weights to the nearest 0.0001 g  
Environmental test chamber or water bath capable of maintaining  $20 \pm 1^{\circ}\text{C}$   
1000 mL test chambers  
Clean filtered seawater  
Deionized water  
Pipets  
Brushes  
Miscellaneous labware (wash bottles, tally counters, culture bowls, etc.)  
500  $\mu\text{m}$  stainless steel sieves  
Aluminum weigh boats  
Holding cups (food grade plastic is acceptable)  
Stir plate and teflon stir bars  
Finely ground TetraMin®  
Centrifuge and centrifuge Teflon® tubes for collecting pore water  
Drying oven capable of maintaining  $60^{\circ}\text{C}$   
Muffle furnace capable of  $550^{\circ}\text{C}$   
Desiccator

### **5.2 Apparatus**

#### **5.2.1 Test Area**

The test area consists of a water bath or a room with constant temperature and appropriate illumination. The facility will be well ventilated and free of fumes.

#### **5.2.2 Lighting**

Continuous overhead lighting will be at 50-100 foot-candles (550-1050 Lux).

#### **5.2.3 Test Chambers**

1000 mL glass beakers with a 10 cm internal diameter covered with a petri dish.

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## **6.0 PROCEDURE**

### **6.1 Preparation**

#### **6.1.1 Labware Preparation**

Labware is described as any plastic or glass material used in the laboratory that will come into contact with any of the test substances or organisms in this evaluation. Labware must be cleaned prior to use. Labware will first be soaked in deionized water then scrubbed with a brush on all surfaces using non-phosphate detergent in deionized water. Alconox® is a widely used established brand of detergent used in laboratory applications. The clean materials will then be rinsed three times with running deionized water. Labware will then be allowed to soak in a 10% hydrochloric acid bath and afterwards rinsed three times with deionized water. Glass labware will also receive a solvent rinse with reagent grade acetone, and finally rinsed three times with deionized water. Some plastic labware is not resistant to solvents and may be damaged by acetone. Plastic labware such as Teflon can receive a solvent rinse, but all other plastics should be investigated prior to solvent rinsing.

#### **6.1.2 Dilution Water Preparation**

Natural seawater will be obtained from North Hood Canal, sand filtered, and filtered to 0.45µm. Seawater will be adjusted as necessary to maintain a target test salinity. Salinity should be lowered with the addition of high purity deionized water or increased with the addition of bioassay grade sea salts or brine.

#### **6.1.3 Test Organism Care and Acclimation**

Upon receipt, salinity and temperature of water in shipping containers should be measured. If salinity is more than 2 ppt different from the target test salinity of 28 ppt then the salinity should be adjusted (no more than 3 ppt daily). If salinity is outside the range of 15 to 35 ppt, then test animals may be possibly stressed and the supplier should be notified to provide a new batch of test organisms. Temperature should be allowed to equilibrate to test temperature prior to removing animals from shipping containers. If temperature of shipping containers is outside the range of 15 to 25°C then a new batch of test organisms may be required. Animals should be held for at least 24 hours prior to testing and may be fed during holding period.

If animal health is suspect upon receipt (e.g. over 10% of number received dead, animals behaving strangely or diseased), notify the laboratory manager who will assess whether to notify the supplier and order replacements. If more than 10% of the organisms die in the 48h prior to testing, the entire batch is discarded, and a new batch is ordered. If the acclimation process is repeated with a new group of test organisms and excessive mortality occurs, an alternative source of dilution water should be used.

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## **6.2 Primary Task**

### **6.2.1 Test Sediment Addition**

Test sediment will be prepared using labware cleaned according to Section 6.1.1, pre-cleaned labware of a disposable nature, or non-toxic food grade plastic.

If pre-sieving of sediment is required to exclude large material or potential predators they may be press sieved (no water) through a clean stainless steel sieve (2 mm mesh).

One day prior to test initiation test sediment, reference, and control sediment should be added to the test chambers. Sediment should be thoroughly homogenized prior to addition to the test chambers. A subsample should be analyzed for pore water ammonia. Approximately 2 cm of sediment should be added to each of the 5 replicate containers and applicable surrogate chambers. Once sediment has been added, clean filtered seawater should be added up to the 950-mL mark at a salinity of 28 ppt. Water should be added to ensure minimal disturbance of test sediments. Test chambers should be aerated at approximately 100 bubbles/minute under test temperature and photoperiod regime. The system should be left overnight with gentle aeration to allow suspended particles to settle and an equilibrium to be established between sediment and overlying water before the organisms are added.

### **6.2.2 Reference and Control Sediment Test**

During this 20-day toxicity test with *Neanthes arenaceodentata* and a test sediment, a reference substance will be used to provide a site-specific basis for comparison of potentially toxic and non-toxic conditions.

### **6.2.3 Test Organism Addition**

For test initiation, worms should be selected at random from a large culture dish(es) that contains all of the shipped animals. Animals should be added in order of replicate number, not treatment, to ensure an equal distribution of selected animals across treatments (i.e., so that animals selected initially aren't all in a single treatment and animals selected at the end aren't all in a single treatment). Transfer of animals to the test chambers is accomplished by gently drawing one worm into the wide end of a Pasteur pipette and adding the organism directly to the test chamber just above the water's surface to prevent cross-contamination. The number of animals added will be tracked by a cell counter operated by the person adding the worms. As animals are added to the test chamber, test chambers should be marked. Test chambers should be observed within one hour of addition. Worms demonstrating non-burrowing behavior may be replaced, if the observer believes the behavior results from factors other than sediment toxicity.

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During test initiation, five worms should be assigned to an additional 3 holding cups for initial calculated individual weight measurements. Worms for these measurements should be selected at random from the culture dish and should be collected at regular intervals during initiation of the test so as not bias the initial size measurements.

#### **6.2.4 Test Initiation**

The test is initiated when test organisms are distributed completely to each test chamber.

To make initial weight measurements, individual animals should be gently scooped onto a small brush, rinsed briefly in deionized water, blotted dry on a Kimwipe and transferred onto a pre dried, pre weighed, pre marked (number etched into pan prior to pre weighing) aluminum pan (2x2 cm piece of aluminum foil). All five worms from one holding cup should be placed onto a foil weigh boat. Fold pans over to prevent loss of animals over the course of drying. Oven dry worms and pans at 60°C for 24 hours prior to weighing. Remove pans/worms from the oven and place in a desiccator for approximately 1 hour to cool to room temperature. All weight measurements must be made on a balance that can be measured to the nearest 0.01 mg.

An initial ash-free dry weight (AFDW) measurement may also be desired on the worms if this endpoint is included in the final test weight determinations. After obtaining the dry weight data, each of the weigh boats is then dried in a muffle furnace heated to 550°C for 2 hours in order to determine ashed weights. The ashed boats are again weighed to 0.01 mg and the ashed weight is subtracted from the dry weight to calculate the AFDW.

#### **6.2.5 Test Maintenance**

Feed worms every 48 hours. TetraMin® should be provided at approximately 8 mg (dry weight) per juvenile *Neanthes* (40 mg per test chamber).

Overlying water should be renewed every three days (total of six renewals). Approximately one third of the overlying water volume should be exchanged at each renewal.

#### **6.2.6 Test Measurements**

Data are recorded on data sheets.

**Water Quality.** A daily record of test room or water bath temperatures and test chamber aeration should be made. Water quality measurement should be made prior to renewals. Record temperature, salinity, dissolved oxygen, and pH in one randomly selected test chamber per treatment or a designated water quality surrogate chamber.

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**Biological.** Response criteria indicating toxicity of test sediment include mortality, sublethal and chronic effects. A sublethal effect is the emergence from highly toxic sediment during the course of the test. Chronic effects are monitored by comparing the differences in dry weight or AFDW between test sediments and reference sediments (or control treatment when appropriate). Response criteria will be monitored in a "blind" fashion, that is, the observer will have no knowledge of the treatment of the sediment in the test chambers.

**Mortality.** At test termination (Day 20), all sediment from each individual chamber should be sieved through a 500 µm sieve to collect surviving organisms. Gently rinse sediment through sieve using 26 - 30 ppt salinity seawater. Gently remove animals from the sieve using a camel hair brush taking care not to damage the animal. Once removed, the animal should be placed into a labeled holding container containing clean filtered seawater (26 - 30 ppt) at room temperature. Record whether animal recovered from each test chamber is surviving, dead, or missing (for purposes of calculations all missing animals are assumed to be dead).

**Growth (Dry Weight).** Growth is measured by the dry weight of the surviving test worms within a replicate. The results are compared with the weight of the worms at the beginning of the test and with the control(s) and the test concentrations of sediment. Each surviving animal is removed from its holding cup, rinsed briefly in deionized water (< 5 seconds) blotted dry on a Kimwipe, and then placed onto a pre dried, pre weighed, pre labeled weigh boat. The aluminum foil boat should be folded over to prevent the loss of the animal during drying. Note weigh boats should be handled with forceps only. Oven dry animals at 60°C for 24 hours, remove animals and boats from oven and allow to come to room temperature in a desiccator prior to weighing on a microbalance to the nearest 0.01 mg. Subtract boat weight from total weight to obtain measured dry weight value of surviving worms.

**Growth Modification (Ash-Free Dry Weight).** The purpose of this modification is to account for the weight of sediment contained in the gut of the worms during the drying process. Worms reared under similar conditions and life history, but exposed to different grain size sediment, may express significantly different dry weights due to the contribution of heavier gut material of the worms maintained in sandy (heavier particles) sediment. This discrepancy has the potential to lead to Type II errors, where significant differences are found between test treatments, when none actually exist. The procedure below is a tool to estimate the actual contribution of gut content to the overall weight of the animals. A procedure defined as "ashing" is employed to heat the worm tissue at high temperatures until all that is left behind is solid inorganic material.

At the termination of the 20-day survival and growth test, sediment from each test chamber is sieved through a 0.5-mm screen and all recovered polychaetes are transferred into a plastic cup. Survival is recorded and worms are rinsed with deionized water and placed in pre-ashed, pre-weighed aluminum boats and dried in a gravimetric

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oven at 60°C for at least 24 hours. Each weigh-boat is removed from the oven, cooled in a desiccator for approximately 30 minutes, and then weighed on an analytical microbalance to 0.01 mg. Each of the weigh boats is then dried in a muffle furnace heated to 550°C for 2 hours in order to determine ashed weights. The ashed boats are again weighed to 0.01 mg and the ashed weight is subtracted from the dry weight to calculate the AFDW. Both the dry weight and the AFDW are used to determine individual worm weight and growth rates.

The 20-day average individual dry weight (or AFDW) in each exposure chamber is recorded and the mean and standard deviation calculated for each treatment.

### 6.3 Post-task

#### Results Needed:

- Percent mortality for each treatment
- Mean dry weight per individual for each treatment
- Ash-Free dry weight (AFDW) per individual for each treatment (program specific, if desired)
- Mean water quality values by treatment
- LC<sub>50</sub> and 95% confidence limits (for ref. tox.)
- Tables showing biological, chemical, and physical data

In screening tests, the responses of worms in collected test sediments are compared to control and reference site sediments.

### 6.4 Reporting

The report may include, but will not be limited to, the following:

- Name and address of the laboratory conducting the study, and dates on which the study was initiated and completed.
- The name of the Study Director, other scientists or professionals, and supervisory personnel involved in the study.
- Objectives as stated in the protocol.
- A description of the methods used.
- Transformations, calculations, or operations performed on the data, a summary and analysis of the data, and a statement of the conclusion drawn from the analysis.
- The test substance identified by code number and the date each sample was used.

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*Neanthes arenaceodentata***

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- The number of organisms used in the study.
- Concentrations of exposure and exposure method.
- Any circumstances that may have affected the quality or integrity of the data, including deviations from test protocols or Standard Operating Procedures.
- The location where raw data and the final report will be stored.
- Additions or corrections to a final report will be in the form of an amendment by the Study Director. The amendment will clearly identify that part of the final report that is being altered and the reason(s) for the alteration(s). The amendment will be signed and dated by the Study Director.

The master copy of the final report will be signed and dated by the Study Director.

## **7.0 HEALTH AND SAFETY CONSIDERATIONS**

Proper laboratory protection, including lab hood or ventilation system, lab coat, closed-toe shoes, gloves and safety glasses, is required when working with chemicals and unprocessed samples.

Refer to the Port Gamble Laboratory's Health and Safety Plan at [S:\Health and Safety](#) for procedures to ensure safe operation in the laboratory and for contingency plans in the event of an accident or emergency.

For specific chemical health and safety information, refer to the Safety Data Sheet log.

## **8.0 PERSONNEL**

Any laboratory personnel demonstrating competence with this method may perform the procedure.

## **9.0 QUALITY ASSURANCE REQUIREMENTS (ACCEPTANCE CRITERIA)**

This study will be conducted according to the Standard Operating Procedures which are in effect during the time the study is being performed. In the case where there is a conflict between the other SOPs and this protocol, the protocol will be the definitive procedure.

Usually tests would be unacceptable if one or more of the following occurred:

- More than 10% of the control organisms die.
- All test chambers were not identical.

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**Title: 20-Day Chronic Growth and Survival Test with  
*Neanthes arenaceodentata***

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- Treatments were not randomly assigned to test chambers.
- Test organisms were not randomly or impartially distributed to test chambers.
- All test animals were not from the same population, were not all of the same species, or were not of acceptable quality.
- Reference sediment and controls were not included in the test.
- Temperature, DO, pH, salinity, and ammonia were not measured, or were not within acceptable range.
- Aeration to the test chamber was off for an extended time such that the DO levels dropped to less than 4.6 mg/L.
- Response criteria were not monitored in a blind fashion.

## 10.0 REFERENCE DOCUMENTS

ASTM. 2012. Guide for conducting Sediment Toxicity Test with Marine and Estuarine Polychaetous Annelids. Standard Guide #E-1611-00(Reapproved 2007). American Society for Testing and Materials, Philadelphia, P A.

Puget Sound Water Quality Authority. Revised July 1995. "Recommended Guidelines for Conducting Laboratory Bioassays on Puget Sound Sediments." Prepared for U.S. EPA Region 10, Office of Puget Sound. Seattle, W A.

## 11.0 APPENDIX OF CHANGE

- 08/20/15 Hand-written change to reflect criteria: if >5% mortality 48hrs preceding test, organisms should be replaced
- 11/12/15 Updated logo, corrected microbalance to nearest gram, and changed test organism acclimation section to reflect a 10% mortality threshold when assessing the organism's health during rounds
- 05/23/16 Added "uncontrolled" statement to SOP footer and updated Health and Safety section
- 05/09/17 Updated health and safety information, removed branding, added review documentation section. Added "proprietary information" statement in footer. Updated information on animal health upon receipt in section 6.1.3.

## STANDARD OPERATING PROCEDURE

### SPT AND SPLIT SPOON SAMPLING

#### Introduction

Split spoon sampling is generally used to collect disturbed soil samples of 18 or 24 inches in length. A series of consecutive cores may be extracted with a split spoon sampler to give a complete soil column profile, or an auger may be used to drill down to the desired depth for sampling, providing samples at discrete depth intervals. During discrete sampling, the split spoon is driven to its sampling depth through the bottom of the augured hole and the core extracted. A split-spoon sample is ideal for collecting subsurface geotechnical data, including relative soil strength information through the standard penetration test (SPT). When split spoon sampling is performed to gain geologic information, all work should be performed in accordance with ASTM D1586, "Standard Test Method for Penetration Test and Split-Barrel Sampling of Soils". This SOP describes the SPT and collection of soil samples using the split spoon.

#### Standard Penetration Test

The SPT is an approximate measure of soil density and consistency. To be useful, the results must be used with engineering judgment in conjunction with other tests. The SPT (ASTM D1586) is used to obtain disturbed soil samples. This test employs a standard 2-inch outside diameter<sup>1</sup> split-spoon sampler. Using a 300-pound hammer, free-falling 30 inches, the sampler is driven into the soil for 18 inches. The number of blows required to drive the sampler the last 12 inches only is the Standard Penetration Resistance. This resistance, or blow count, measures the relative density of granular soils and the consistency of cohesive soils. The blow counts are plotted on the boring logs at their respective sample depths.

Soil samples are recovered from the split-barrel sampler, field classified, and placed into watertight jars or double bagged in ziplock bags. They are then shipped to the geotechnical laboratory for further testing.

#### *In the Event of Hard Driving*

Occasionally very dense materials preclude driving the total 18-inch sample. When this happens, the penetration resistance is entered on logs as follows:

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<sup>1</sup> Different sized samplers may be used, including oversized split spoons where sample volume requirements dictate collecting more soils. In all cases, the size of the split spoon sampler shall be recorded in field records so that SPT blowcounts can be corrected, if necessary, for non-standard split spoon sizes.

**Penetration less than six inches.** The log indicates the total number of blows over the number of inches of penetration.

**Penetration greater than six inches.** The blow count noted on the log is the sum of the total number of blows completed after the first 6 inches of penetration. This sum is expressed over the number of inches driven that exceed the first 6 inches. The number of blows needed to drive the first 6 inches are not reported. For example, a blow count series of 12 blows for 6 inches, 30 blows for 6 inches, and 50 (the maximum number of blows counted within a 6-inch increment for SPT) for 3 inches would be recorded as 80/9.

## **Required Information on Boring Logs**

Logs shall include the following information, at a minimum, to describe the drilling work:

1. The sample station identification
2. Names of field personnel collecting and handling the samples
3. Type of sampling equipment used (i.e. split spoon inside and outside diameter; hammer weight; free fall height; hammer deployment method)
4. Date and time at the start and completion of each boring location
5. Total depth of boring penetration below ground surface or mudline
6. Depth of water and time of measurement for barge-based drilling
7. Date and time of collection of each sample
8. Driller observations regarding changes in subsurface conditions, and depth of drilling equipment at the time of observation (e.g. gravelly drill action, hard penetration, etc.)
9. Observations made during sample collection, including weather conditions, complications, and other details associated with the sampling effort
10. Length and depth intervals of each sample and measured recovery
11. Qualitative notation of apparent resistance during driving
12. Any deviation from the approved SAP

## **Split Spoon Sample Collection and Processing**

The following procedures are used for collecting soil samples with a split spoon:

1. Assemble the sampler by aligning both sides of barrel and then screwing the drive shoe (with catcher) on the bottom and the head piece on top.
2. Place the sampler in a perpendicular position on the sample material. Where a drill rig is used, this step is performed by the drilling contractor.

3. Use the SPT hammer to drive the tube. Do not drive past the bottom of the sample length. Where a drill rig is used, this step is performed by the drilling contractor.
4. Record in the site logbook or on field data sheets the length of the tube used to penetrate the material being sampled, and the number of blows required to obtain this depth, in 6-inch depth intervals
5. Withdraw the sampler, and open by unscrewing the bit and head and splitting the barrel. The amount of recovery and soil type should be recorded on the boring log.
  - a. If a split sample is desired, a cleaned, stainless steel knife should be used to divide the tube contents in half, longitudinally.
  - b. The split spoon sampler is typically available in 2- and 3-1/2-inch diameters. A larger barrel may be necessary to obtain the required sample volume.
6. Record soil description on the field log, and place samples in labeled, watertight containers.
7. Store sample in a dry location outside of direct sunlight.

### **Split Spoon Sample Logging**

Split spoon samples will be logged on-site by an experienced field geologist or geotechnical engineer. Prior to sub-sampling, a description of each sample will be recorded on a standard boring log. The following parameters will be noted:

1. Sample recovery
2. Physical soil description in accordance with the Unified Soil Classification System (includes soil type, moisture, density/consistency of soil, color)
3. Odor (e.g., hydrogen sulfide, petroleum)
4. Visual stratification, structure, and texture
5. Vegetation and debris (e.g. woodchips or fibers, concrete, metal debris)
6. Biological activity (e.g., detritus, shells, tubes, bioturbation, live or dead organisms)
7. Presence of oil sheen

Standard terminology for field logs is attached to this SOP.

## Sample Description Key

Classification of soils in this report is based on visual field and laboratory observations which include density/consistency, moisture condition, grain size, and plasticity estimates and should not be construed to imply field nor laboratory testing unless presented herein. Visual-manual classification methods of ASTM D 2488 were used as an identification guide.

Soil descriptions consist of the following:

Density/consistency, moisture, color, minor constituents, MAJOR CONSTITUENT, additional remarks.

## Density/Consistency

Soil density/consistency in borings is related primarily to the Standard Penetration Resistance. Soil density/consistency in test pits is estimated based on visual observation and is presented parenthetically on the test pit logs.

SAND or GRAVEL Density	Standard Penetration Resistance (N) in Blows/Foot	SILT or CLAY Consistency	Standard Penetration Resistance (N) in Blows/Foot	Approximate Shear Strength in PSF
Very loose	0 - 4	Very soft	0 - 2	< 250
Loose	4 - 10	Soft	0 - 4	250 - 500
Medium dense	10 - 30	Medium stiff	4 - 8	500 - 1,000
Dense	30 - 50	Stiff	8 - 15	1,000 - 2,000
Very dense	>50	Very stiff	15 - 30	2,000 - 4,000
		Hard	>30	> 4,000

## Moisture

Dry	Little perceptible moisture
Damp	Some perceptible moisture, probably below optimum
Moist	Probably near optimum moisture content
Wet	Much perceptible moisture, probably above optimum

## Minor Constituents





Estimated Percentage

Not identified in description	0 - 5
Slightly (clayey, silty, etc.)	5 - 12
Clayey, silty, sandy, gravelly	12 - 30
Very (clayey, silty, etc.)	30 - 50




## Legends

### Sampling Test Symbols

#### BORING SAMPLES

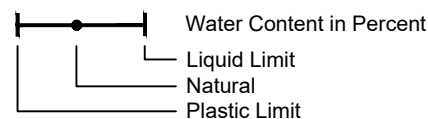
-  Split Spoon
-  Shelby Tube
-  Cuttings
-  Core Run
- \* No Sample Recovery
- P Tube Pushed, Not Driven

#### TEST PIT SAMPLES

-  Grab (Jar)
-  Bag
-  Shelby Tube

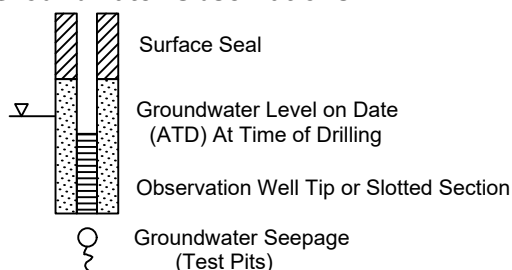
### Test Symbols

- GS Grain Size
- Comp Composite
- Chem Chemistry
- VST Vane Shear Test
- BD Bulk Density
- UU Triaxial Unconsolidated Undrained
- TCU Triaxial Consolidated Undrained
- TCD Triaxial Consolidated Drained
- QU Unconfined Compression
- DS Direct Shear
- K Permeability
- PP Pocket Penetrometer - Approximate Compressive Strength in TSF
- TV Trovane - Approximate Shear Strength in TSF
- AL Atterberg Limits



- PID Photoionization Detector Reading
- Note: additional observation modifiers to be added as appropriate, including approximate amount (e.g. trace debris) at depth interval where encountered

## Groundwater Observations



## **STANDARD OPERATING PROCEDURE**

### **THIN WALL SAMPLING**

#### **Introduction**

Undisturbed<sup>1</sup> soil sampling is performed using a thin wall (a.k.a. Shelby tube) sampler advanced by hand, or hydraulically pushed from a land- or water-based drill rig. Thin wall sampling is generally used to collect undisturbed soil cores of 24 to 36 inches in length. A series of consecutive cores may be extracted with a thin wall sampler to give a complete soil column profile, or an auger may be used to drill down to the desired depth for sampling for a discrete sample interval. In the case of discrete interval sampling, the thin wall sampler is pushed to its sampling depth through the bottom of the augured hole and the core extracted. A thin wall sampler is ideal for collecting relatively undisturbed subsurface geotechnical samples for advanced geotechnical laboratory testing. When thin wall sampling is performed to gain geologic information, all work should be performed in accordance with ASTM D1587, "Thin-Walled Tube Sampling of Soils for Geotechnical Purposes". This SOP describes the collection of soil samples using the thin walled sampler.

#### **Thin Wall Sample Collection and Processing**

The following procedures are used for collecting soil samples with a thin-walled sampler:

1. Assemble the sampler by attaching the driving head to the sampling tube.
2. Place the sampler in a perpendicular position on the sample material. Where a drill rig is used, this step is performed by the drilling contractor.
3. Use a smooth continuous push to advance the tube. Do not drive past the bottom of the sample length. Where a drill rig is used, this step is performed by the drilling contractor.
4. Record in the site logbook or on field data sheets the length of the tube used to penetrate the material being sampled, as well as the detailed information described in this SOP.
5. Withdraw the sampler and remove the drive head. The amount of recovery and soil type should be measured recording the depth to soil from both the top and bottom of the sample tube. Note any aberrations such as rocks or other objects visible in the drive end of the sampler, or any visible damage to the sample tube.
6. Fill any voids in the sample tube with packing material such as bubble wrap or wax.
7. Tightly seal both ends of the sample tube, and visually indicate the top of the tube with markings on the outside of the tube.

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<sup>1</sup> The term "undisturbed" is relative for soil sampling. All soil sampling induces some disturbance to the sample. Thin-walled sampling induces the least disturbance, compared to other methods such as standard penetration test (SPT) split-spoon sampling.

8. Store sample upright in a protected and dry location outside of direct sunlight.

## **Required Information on Boring Logs**

Logs shall include the following information, at a minimum, to describe the sampling work:

1. The sample station identification
2. Names of field personnel collecting and handling the samples
3. Type of sampling equipment used
4. Date and time at the start and completion of each boring location
5. Total depth of boring penetration below ground surface or mudline
6. Depth of water and time of measurement for barge-based drilling
7. Date and time of collection of each sample
8. Driller observations regarding changes in subsurface conditions, and depth of drilling equipment at the time of observation (e.g. gravelly drill action, hard penetration, etc.)
9. Observations made during sample collection, including weather conditions, complications, and other details associated with the sampling effort
10. Length and depth intervals of each sample and measured recovery
11. Qualitative notation of apparent resistance during driving
12. Any deviation from the approved SAP

## **Thin Wall Sample Handling**

Thin wall tube samples do not allow for direct observation or logging in the field. When recovered from the boring, the tubes will be measured for amount of recovery and checked to ensure the tube was not dented or damaged while driving or removing. The tubes will then be quickly cleaned, sealed with a plastic cap and duct tape on both ends, and labeled with boring name, sample name, date, approximate depth, and the location of the top of the sample with respect to the orientation it was removed from the subsurface. Every effort will be made to store and transport the thin wall sample tubes with minimal disturbance in the upright, vertical position.

Standard terminology for field logs is attached to this SOP.

## Sample Description

Classification of soils in this report is based on visual field and laboratory observations which include density/consistency, moisture condition, grain size, and plasticity estimates and should not be construed to imply field nor laboratory testing unless presented herein. Visual-manual classification methods of ASTM D 2488 were used as an identification guide.

Soil descriptions consist of the following:

Density/consistency, moisture, color, minor constituents, MAJOR CONSTITUENT, additional remarks.

### Density/Consistency

Soil density/consistency in borings is related primarily to the Standard Penetration Resistance. Soil density/consistency in test pits is estimated based on visual observation and is presented parenthetically on the test pit logs.

Density SAND or GRAVEL	Standard Penetration Resistance (N) in Blows/Foot	SILT or CLAY Consistency	Standard Penetration Resistance (N) in Blows/Foot	Approximate Shear Strength in TSF
Very loose	0 - 4	Very soft	0 - 2	<0.125
Loose	4 - 10	Soft	2 - 4	0.125 - 0.25
Medium dense	10 - 30	Medium stiff	4 - 8	0.25 - 0.5
Dense	30 - 50	Stiff	8 - 15	0.5 - 1.0
Very dense	>50	Very stiff	15 - 30	1.0 - 2.0
		Hard	>30	>2.0

### Moisture

Dry	Little perceptible moisture
Damp	Some perceptible moisture, probably below optimum
Moist	Probably near optimum moisture content
Wet	Much perceptible moisture, probably above optimum

### Minor Constituents





Estimated Percentage

Not identified in description	0 - 5
Slightly (clayey, silty, etc.)	5 - 12
Clayey, silty, sandy, gravelly	12 - 30
Very (clayey, silty, etc.)	30 - 50




## Legends

### Sampling Test Symbols

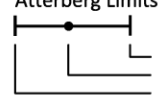
#### BORING SAMPLES

	Split Spoon
	Shelby Tube
	Cuttings
	Core Run
*	No Sample Recovery
P	Tube Pushed, Not Driven

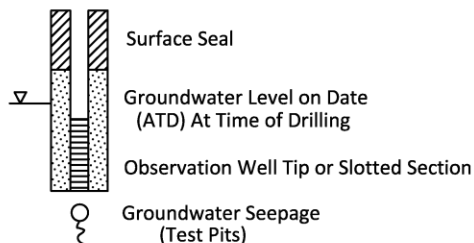
#### TEST PIT SAMPLES

	Grab (Jar)
	Bag
	Shelby Tube

### Test Symbols

GS	Grain Size
Comp	Composite
Chem	Chemistry
NS	No Sheen
SS	Slight Sheen
MS	Moderate Sheen
HS	Heavy Sheen
TCD	Triaxial Consolidated Drained
QU	Unconfined Compression
DS	Direct Shear
K	Permeability
PP	Pocket Penetrometer Approximate Compressive Strength in TSF
TV	Torvane Approximate Shear Strength in TSF
CBR	California Bearing Ratio
MD	Moisture Density Relationship
AL	Atterberg Limits  Water Content in Percent
PID	Photoionization Detector Reading
CA	Chemical Analysis
DT	In Situ Density Test

### Groundwater Observations



## **STANDARD OPERATING PROCEDURE**

### **CONE PENETROMETER TESTING**

#### **Introduction**

Cone Penetrometer Testing (CPT) is generally used to develop a continuous profile of subsurface soil/sediment geotechnical characteristics. CPT penetration tip resistance, side friction, and pore water pressure are continuously measured as the instrumented probe is advanced into the subsurface. These three parameters are further processed to develop a range of geotechnical engineering soil classification parameters using industry-standard protocols for interpreting CPT data. This SOP describes subsurface data collection using the CPT.

#### **CPT Data Collection and Processing**

The electric piezocone penetrometer test procedure involves hydraulically pushing in instrumented cylindrical rod into the subsurface at a constant rate of two centimeters per second and subsequently monitoring soil and pore fluid response near the conical tip. The cylindrical rod houses a pressure transducer and load cells which, during probing, measure the parameters indicated above. The results are used with engineering judgment in conjunction with other tests, preferably the SPT procedure, which allows soil sample collection for direct comparison purposes. CPT tests are performed in general accordance with procedures outlined in ASTM D 5778, Standard Test Method for Electronic Friction Cone and Piezocone Penetration Testing of Soils.

The cone system is mounted on heavy equipment to provide the necessary reaction for the applied loads. The cone tip has a surface area of about 10 square centimeters ( $\text{cm}^2$ ) and an angle of 30 degrees from the axis. The friction sleeve has a surface area of about 150  $\text{cm}^2$ . Prior to testing, a plastic filter element, which has been saturated under vacuum in glycerin, is placed behind the cone tip. This filter element transmits pore pressures to the transducer. Load cells measure end resistance on the tip and frictional resistance on the friction sleeve. As the cone penetrates the soil, measurements are continuously recorded on a portable computer at depth increments of about 5 centimeters.

The classification method used to develop an interpreted soil profile is based on normalized parameters provided by the piezocone, as there are no soil samples collected with a penetrometer system of this type. The relationship between the cone tip resistance and friction ratio, which has been normalized for soil overburden stresses, can be established to predict soil behavior (e.g. Sabatini et. al. 2002).

Commercial software is often used to process raw CPT data and to provide geologic and geotechnical engineering interpretations of subsurface conditions. Some CPT contractors provide a complete report, with data interpretation as part of their deliverable. In other cases, CPT contractors may provide interpreted data and raw data for independent interpretation.

Independent interpretation of raw CPT data can be performed by commercial software programs. At Anchor QEA, the software package CPeT-IT by Geologismiki is used for raw CPT data interpretation, and CLiq by Geologismiki is used for liquefaction evaluations of CPT data.

Where commercial software is not used to process raw CPT data, the methods described in Sabatini et. al. (2002) will be used to process CPT data for use in geotechnical engineering evaluations.

## References

Sabatini, P.J., Bachus, R.C., Mayne, P.W., Schneider, J.A., and Zettler, T.E., 2002. *Geotechnical Engineering Circular No. 5, Evaluation of Soil and Rock Properties, Report No. FHWA-IF-02-034*. U.S. Department of Transportation, Federal Highway Administration, Washington, D.C.

## **STANDARD OPERATING PROCEDURE**

### **DYNAMIC CONE PENETROMETER TESTING**

#### **Introduction**

Dynamic Cone Penetrometer (DCP) testing is generally used to develop a continuous profile of subsurface blowcounts indicating relative density of soils, using handheld equipment for locations with difficult access. DCP blowcounts can be correlated to standard penetration test (SPT) blowcounts for further use in classifying site subsurface soils. This SOP describes subsurface data collection using the DCP.

#### **DCP Data Collection and Processing**

DCP data collection entails advancing a 10-square-centimeter field point fixed to the end of a drive rod that is driven continuously through a series of hammer blows. The blows are delivered to the top of the 1-meter-long drive rod through the raising and dropping of a 35-pound weight at a consistent fall height of 15 inches. The number of blows is recorded for each 10-centimeter interval for the full drive length. Additional 1-meter drive rods are added as needed until refusal or the desired investigation depth is reached. The blow count data obtained are then correlated to an equivalent SPT N-value for each 10-centimeter interval. The SPT N-values are then used to estimate the geotechnical engineering properties of interest.

#### **EQUIPMENT AND SUPPLIES REQUIRED**

- 10-square-centimeter field points (sacrificial)
- 1-meter drive rods with marks at 10-centimeter intervals and thread connections
- Driver hammer attachment with 35-pound weight with minimum 15 inches of throw
- Driving head (threaded connection)
- Mechanical drive rod extractor
- Base plate for drive rod extractor
- Equipment carrying case
- Field logbook, surface soil field collection form, and pens
- Project-specific Field Sampling Plan (FSP) and Health and Safety Plan (HASP)
- Personal protective equipment (safety glasses, steel-toed boots, work gloves, and any other items required by the project-specific HASP)
- Decontamination equipment

## **PROCEDURES**

### **Performing the Exploration**

1. Expose the soil surface by clearing an approximately 1-square-foot area at the sampling site of any surficial detritus that may otherwise create an obstruction
2. Ensure the driving head is threaded onto a 1-meter drive rod
3. Fix the field point to the driving head
4. Place drive rod and field point unit on the ground with field point resting on the desired location of the exploration
5. Connect the driver attachment with the 35-pound hammer
6. Seat the field point by raising and dropping the driver until the field point is fully embedded in the subsurface
7. Begin raising and dropping the driver and count the number of blows required for 10 centimeters of advancement
  - a. Record the blows for each 10-centimeter drive interval; manual adjustments may be needed to maintain verticality of the drive rods
  - b. Record any obstructions/zones of difficult driving or apparent changes in substrate that occur within the 10-centimeter interval, if applicable, on the field data collection form
8. Connect additional drive rods as needed until refusal or the desired drive depth is achieved
  - a. Refusal is assumed to be 50 blows per 10 centimeters or less
  - b. Leave approximately 0.5 meters of stickup of the last rod for extraction
9. After the target depth or refusal is reached, place the base plate near the rod and fix the extractor and begin retrieving the rods
  - a. The drive hammer may need to be removed to allow for extraction of drive rods
  - b. Care must be applied when extracting the rod so as to not drop loose rods back down the hole
10. Decontaminate equipment, if necessary

### **Data Processing**

1. Input the data obtained for each exploration into the data processing spreadsheet, which was developed based on methods described in Triggs and Simpson (1991), attached.
2. The depth, field recorded blow count, and equivalent SPT N-value are reported for the processed data

## Quality Assurance and Quality Control

1. Complete all pertinent field quality assurance (QA)/quality control (QC) documentation, logbooks, sample labels, and field data sheets
  - a. Record any deviations from the specified sampling procedures or any obstacles encountered
2. Record actual exploration locations with field measurements from landmarks or a Differential Global Positioning System (DGPS) recording device
3. Photograph the sample location and document it in the logbook
4. Decontaminate all sampling equipment as appropriate

## References

Triggs and Simpson, 1991. *A Portable Dynamic Penetrometer for Geotechnical Investigations*. 34<sup>th</sup> Annual Meeting, Association of Engineering Geologists. Chicago, IL.

## Attachment

# A PORTABLE DYNAMIC PENETROMETER FOR GEOTECHNICAL INVESTIGATIONS

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Triggs Technologies, Inc., Willoughby Hills, OH 44094

Paul D. Simpson, P.E.,  
University of Akron, Akron, OH 44325

## INTRODUCTION

Most of us are frustrated by our human inability to "see" into the ground. Without special instruments, we can only see, probe, or squeeze the very surface of the ground. Such looks, probes, and squeezes give little reliable information about its ability to support loads because its supporting properties depend upon the soils' strength, deep within the ground.

The authors have developed and tested a lightweight dynamic cone penetrometer which one person can carry to remote locations where he can readily test for strength properties to deep within the soil. A means of injecting fluid into the cone path annulus to reduce parasitic friction dynamic energy loss is described. A logging program which quickly reduces the penetrometer's blow count data to dynamic cone resistances, plots these resistances, and interprets them, is described and illustrated.

Calibration of this dynamic cone penetrometer has been accomplished by the authors' correlations of side-by-side dynamic cone resistances with Standard Penetration Test N values in various soil types. This paper proposes correlations between this penetrometer's dynamic cone resistances and SPT N values.

## THE NEED FOR A PORTABLE SOIL EVALUATION INSTRUMENT

The normal commercial structure needs and generally receives a subsurface exploration including test borings with SPT N values and laboratory strength testing of soil samples. But it is often impractical to use test borings to:

- a. preliminarily explore the subsurface in densely wooded, building covered, swampy, or steep terrain sites.
- b. explore the subsurface for single-family residences.
- c. evaluate earth fill compaction.
- d. verify footing bearing capacities during construction.

## REQUIREMENTS FOR A PORTABLE PENETROMETER

For such a penetrometer to be most useful, it must be usable by a single person; portable, in that it can be carried to test locations; small enough to travel in the trunk of a

car; versatile enough to test any soil type; sensitive enough to differentiate between weak, medium, and strong soils; and simple to interpret.

### DESIGN OF THIS DYNAMIC PENETROMETER

The authors followed these specifications to develop and test a light dynamic cone penetrometer with the characteristics of:

- a. An unmotorized (hand-raised) hammer with a 35 lb weight and a 15 inch drop, to provide the energy for driving a 10 cm<sup>2</sup> projected area cone. This can be transported in a car trunk, carried by one person, and worked by one person to test all but the most consolidated soils. As testing progresses below the first meter, additional 1 meter long sounding rods are screwed onto the rod string.
- b. A fluid injection system, pumps a cellulose/water mixture into the annulus between the 1.4" diameter cone path and the 1.1" diameter rods, minimizing parasitic friction on the rods. Consequently, undiminished hammer energy is transmitted to the cone, allowing confident use of the Dutch Formula to calculate cone resistance ( $q_d$ ). The rods slip out of the "lost point" cones to be pulled from the slurry-filled hole by hand. Cellulose slurry biodegrades.
- c. A simple spread-sheet computer program logs the hammer blows per 10 cm; calculates, plots, and tabulates dynamic cone resistances ( $q_d$ ); and tabulates consistency adjectives.

Unfortunately, this penetrometer, like other dynamic cone penetrometers, does not ascertain the grain size of the tested soils. Consequently, although the data tells much about soils' relative densities, the data does not indicate whether the soils are clay, silt, or sand. Where determination of soil grain sizes is important, that information must be found by some method other than by dynamic cone testing.

### REDUCTION AND LOGGING OF DYNAMIC PENETROMETER DATA

In this paper, dynamic cone resistance,  $q_d$ , is the cone resistance pressure in Kg/cm<sup>2</sup>, calculated by the "Dutch Formula". In that formula,

$$q_d = \frac{M^2 \times H \times N_d}{A_p(M + M' + P_a)10}$$

where,

M = Mass of the hammer = 35# x 0.453 = 15.89 Kg

H = Height of Drop = 15" x 2.54 = 38.1 cm

$N_d$  = Number of blows per 10 cm of drive

$A_p$  = Projected area of the cone = 10 cm<sup>2</sup>

$M'$  = Mass of the driven portion of the hammer = 2.49 Kg

$P_a$  = Mass of the rod string = 3.26 Kg x the number of rods.

This means that the dynamic cone resistance, in Kg/cm<sup>2</sup>, is:

4.44 x  $N_d$  for 1 sounding rod

3.86 x  $N_d$  for 2 sounding rods

3.42 x  $N_d$  for 3 sounding rods

3.06 x  $N_d$  for 4 sounding rods

2.77 x  $N_d$  for 5 sounding rods

2.54 x  $N_d$  for 6 sounding rods  
2.33 x  $N_d$  for 7 sounding rods  
2.16 x  $N_d$  for 8 sounding rods  
2.01 x  $N_d$  for 9 sounding rods  
1.89 x  $N_d$  for 10 sounding rods

The program for logging a dynamic cone penetration test is illustrated in figure 1. The hammer blows per 10 cm are entered. A spread sheet program tabulates these figures, and calculates, tabulates, and plots the dynamic cone resistances. The program also tabulates three columns of consistency adjectives: one for sand, one for silt, and one for clay.

### PENETROMETER CALIBRATION BY CORRELATIONS WITH SPT N VALUES

Since many of us in North America are most comfortable evaluating soil strengths from Standard Penetration Test in-situ test results, we have correlated dynamic cone resistance of the dynamic penetrometer with SPT N values by using both test types in close proximity. In the reduction of the correlation test data, we compared the average of three 10 cm long dynamic cone resistances with the corresponding 12 inch SPT N value.

Soil samples are a byproduct of SPT testing, so we took the opportunity to either visually estimate or test for the mean soil particle size of each SPT test sample. These determinations of  $d_{50}$  in mm were used in our  $q_d/N$  versus  $d_{50}$  plot of Figure 2.

(Robertson, Campanella, and Wightman, 1983) in correlating static cone resistances,  $q_c$  with SPT N, found that  $q_c/N$  increased from approximately 1 for clays to approximately 8 for sands. We have not found a similar increase of  $q_d/N$  to be characteristic of dynamic cone resistances. Perhaps because the dynamic cone penetrometer and the Standard Penetration Test both employ a dynamic penetration force, their relationships are more linear than the relationship of static cone and dynamic SPT.

Figure 3 plots our currently available  $q_d$  versus N correlations. Each plotted point indicates whether it is a clay, silt, or sand soil type. By plotting  $q_d$  directly, rather than the ratio of  $q_d/N$ , one can see how at higher blow counts, the  $q_d/N$  values increase. We believe that this is caused by the relatively low energy of the dynamic penetrometer, compared to the higher energy of SPT.

### CONCLUSIONS

We do believe that this dynamic cone penetrometer, as developed, is a valid instrument for estimating approximate strengths of nearly all soils to reasonable depths. It correlates well with N values of SPT borings. With  $q_d$  values up to 90 Kg/cm<sup>2</sup>,  $q_d/N$  ratios are approximately 3.5.

Two technical reservations of this penetrometer should be understood. The first reservation is that this penetrometer tells nothing about whether a soil is clay, silt, or sand. The second reservation is that when the  $q_d$  values of the cone exceed approximately 90 Kg/cm<sup>2</sup>, SPT N values exceed 25, but are indeterminate.

Most soil evaluation concerns focus on soils with SPT N values less than 15. This penetrometer provides a continuous stream of accurate soil strength data through the entire 0 to 25 range of SPT N values. Consequently, the penetrometer is both usable and most valuable in the soils that require the most concern.

TRIGGS TECHNOLOGIES, INC.  
33977 CHARDON ROAD  
WILLOUGHBY HILLS, OHIO 44094

SHEET #: 1  
FILE #: 1  
STARTED: 4/27/90  
COMPLETED: 4/27/90

\*\*\*\*\*  
WILDCAT DYNAMIC  
CONE PENETROMETER LOG  
\*\*\*\*\*

CREW: ACT  
TEST #: DEMO  
PROJECT: NONE  
LOCATION: HAYES DRIVE

SURFACE ELEVATION:  
HAMMER WEIGHT: 35 LBS.  
CONE AREA: 10 SQ. CM  
HAMMER DROP: 15"

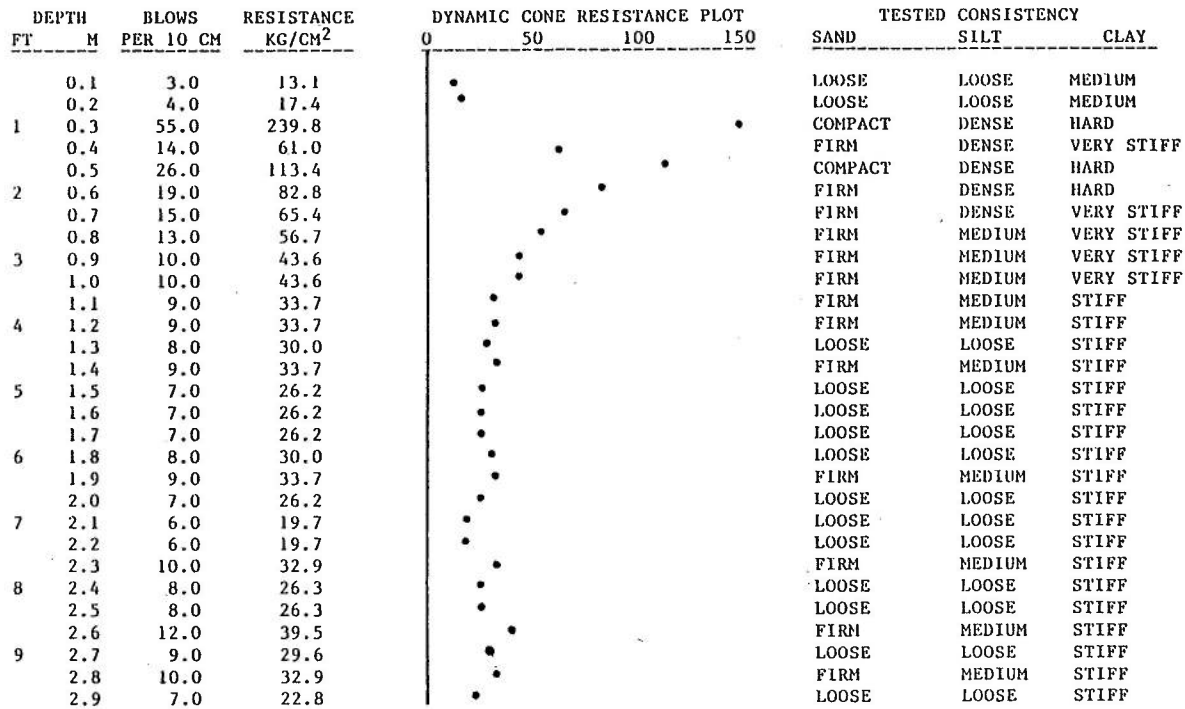


FIGURE 1

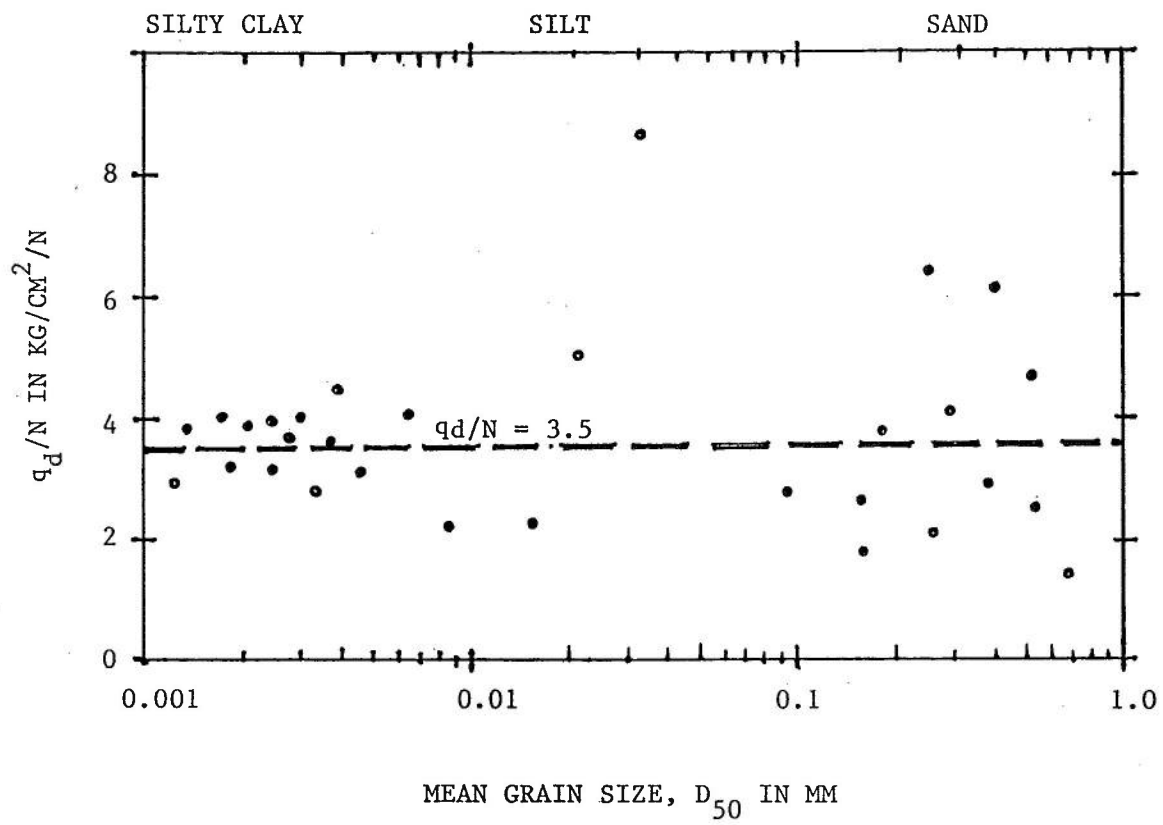


FIGURE 2

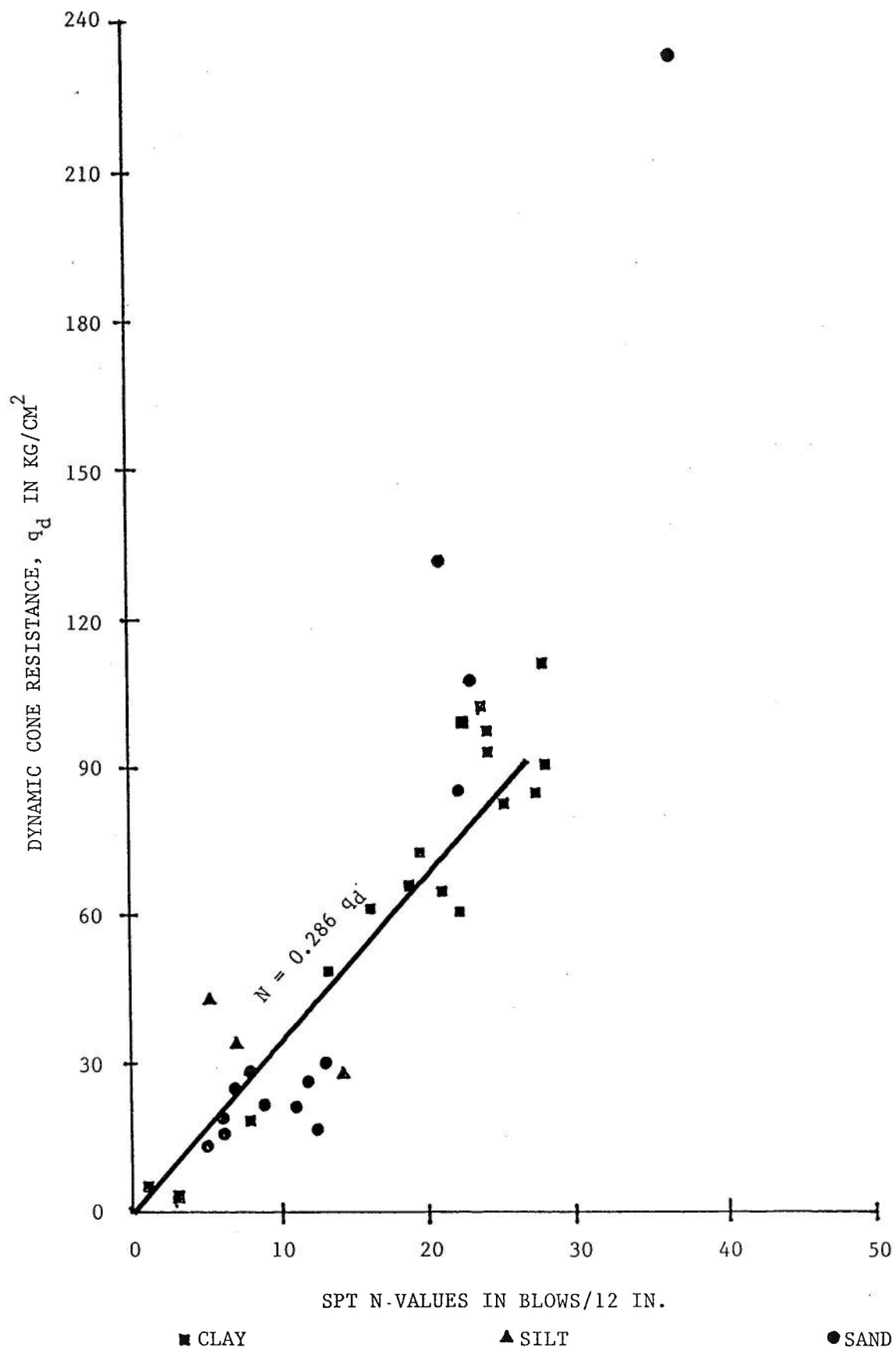


FIGURE 3

## REFERENCE

Robertson, P.K.; Campanella, R.G.; and Whitman, A., "SPT-CPT Correlations," Journal of Geotechnical Engineering, Vol. 109, No. 11, (Nov. 1983), 1449-1459.

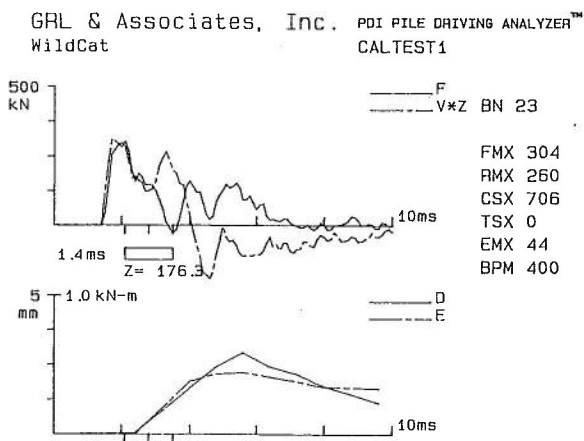
The information below was presented to the 34th Annual AEG Meeting, but was not included in the Proceedings.

### HOW MUCH ENERGY REACHES THE CONE?

ASTM D 4633-86 specifies a method for measuring the portion of a dynamic penetrometer's kinetic energy that is available to drive the tip. GRL and Associates, Inc. instrumented a WILDCAT DYNAMIC CONE PENETROMETER with two accelerometers and two strain transducers and used a pile driving analyser and their CAPWAP program to estimate the effective energy during both easy and hard driving. For easy driving ( $N_d = 7$  blows/10 cm), 82 percent of the kinetic energy was found to be effective. For hard driving ( $N_d = 130$  blows/10 cm), the effective energy was measured as 73 percent. In the arena of dynamic penetrometers, these effective energy percentages show the WILDCAT to be exceptionally efficient. The Force, Velocity, and Energy plots of both the hard and easy driving typical blows, and a photograph of the instrumented penetrometer are shown below.

#### HARD DRIVING

$N_d = 130/10$  cm

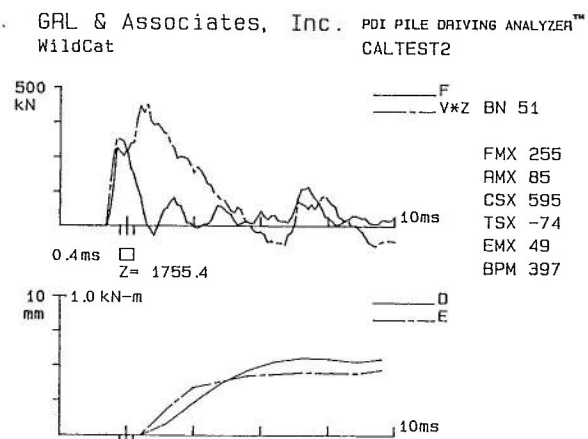


$$\frac{E_{\max}}{E_{KE}} = \frac{44}{60} = 73\%$$



#### EASY DRIVING

$N_d = 7/10$  cm



$$\frac{E_{\max}}{E_{KE}} = \frac{49}{60} = 82\%$$



# INSTRUCTION MANUAL

## FIELD INSPECTION VANE TESTER

### Model H-60

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This product should be installed and operated only by qualified personnel. Its misuse is potentially dangerous. The Company makes no warranty as to the information furnished in this manual and assumes no liability for damages resulting from the installation or use of this product. The information herein is subject to change without notification.

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# 1 APPLICATIONS

The inspection vane borer is used to measure in situ undrained shear strength in clays. It is primarily intended for use in trenches and excavations at a depth not influenced by drying and excavation procedure.

When different sizes of vanes are used, the instrument range is from 0 to 260 kPa. The accuracy of the instrument should be within 10% of the reading.



Figure 1: H-60 vanes in three different sizes

# 2 PRODUCT

## 2.1 DESCRIPTION

The vane tester consists in two parts: the vane with its shaft and the measurement part with its handle.

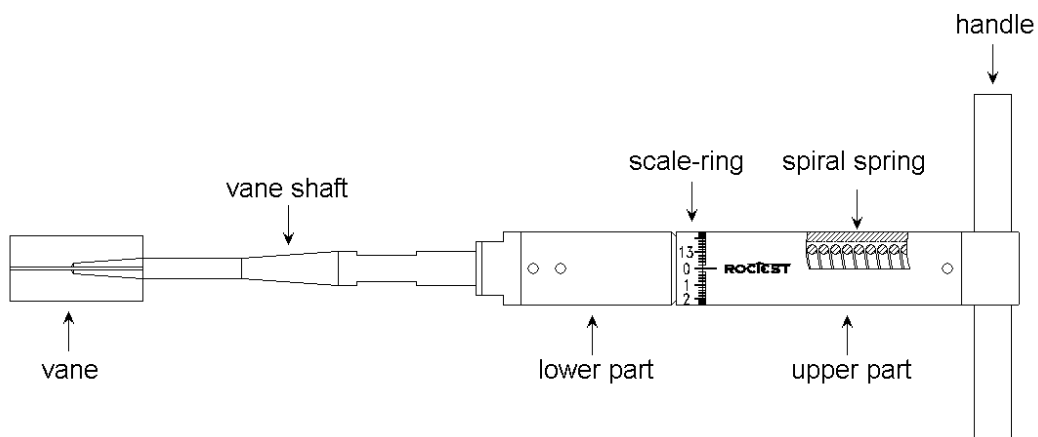


Figure 2: H-60 vane borer

The scale-ring is graduated from 0 to 130 kPa.

Three sizes of four-bladed vanes can be used:

- small: 16 x 32mm
- medium: 20 x 40mm (standard)
- large: 25.4 x 50.8mm

They make it possible to measure shear strength of 0 to 260, 0 to 130 and 0 to 65 kPa respectively.

## 2.2 OPERATION PRINCIPLE

The measurement part of the instrument is a spiral spring. When the handle is turned, the spring deforms. The upper part and the lower part of the instrument get a mutual angular displacement which depends on the torque applied. The shear strength of the soil is obtained by means of a scale-ring.

The lower and upper halves of the instrument are connected by means of threads. The scale is also supplied with threads and follows the upper part of the instrument by means of two lugs. The zero-point is indicated by a line on the upper part. When torque is applied, the scale-ring follows the upper part of the instrument, and when failure in the soil is obtained, the scale-ring will remain in its position due to the friction in the threads.

## 3 OPERATION AND READING PROCEDURES

### 3.1 GENERALITIES

The vane blades are soldered to a shaft which again is extended by one or more 49 cm long rods. The connection between the shaft-rods and the instrument is made by threads. To make the connections as straight as possible, the rods have to be screwed tight together and threads cleaned for dirt.

In clays with shear strength of 260 kPa, a force of about 40 to 50 kilos is required to press the vane down into the soil. The shaft is designed to take this force, but if extension rods are used, precautions against buckling are required.

### 3.2 GENERAL PROCEDURES

1. Connect required vane and extension rods to the inspection vane instrument.

*Note: While screwing vane or rods to instrument, hold onto lower part.*

2. Push vane into the ground to the required position.

*Note: Do not twist inspection vane during penetration.*

3. Make sure that the scale-ring is set to zero-position.

4. Turn handle clockwise.

*Note: Turn as slowly as possible with constant speed.*

**Be careful not to turn the handle over 360°, otherwise the spring inside the instrument may be permanently damaged.**

5. When the lower part follows the upper part around or even falls back, failure and maximum shear strength is obtained in the soil at the vane.
6. Holding handle firmly, allow it to return to zero-position.

*Note: Do not allow the handle to spring back uncontrolled.*

7. Note the reading on the graduated scale.

*Note: Do not touch or in any way disturb the position of the graduated ring until the reading is taken.*

8. Write down the reading together with position of hole and depth.
9. Turn the graduated scale anti-clockwise back to zero-position.
10. To determine the remoulded shear strength, the following procedure is used:
  - Turn the vane quickly at least 25 revolutions. Do not turn using handle. Turn using wrenches provided.
  - Zero the scale and take at least two measurements by turning the instrument as slowly as possible.
  - The minimum value is considered the correct one.
11. Push the vane down to next position. If necessary, screw on another extension rod.
12. Repeat the above measurement procedure (steps 3 to 10).
13. When the last reading is taken, pull the vane up. If soil is comparatively soft, this can be done by hand, gripping the handle. In harder soils, some mechanical device might be necessary. It is then advisable to connect this device directly to the connection rods (not to the instrument).

### 3.3 SPECIAL PROCEDURES

When measuring the shear strength at greater depths, the friction between soil and extension rods can be appreciable, and must be taken into consideration.

To measure the friction, extension rods and a vane-shaft without vane (dummy) are pushed into the ground to the depths required for shear force measurements. The torque due to friction is then measured in the same way as when using vanes (steps above from 3 to 9). The friction torque thus obtained is used to evaluate the actual shear strength from the measured shear strength.

To penetrate through firm layers a pre-boring using a rod with the same diameter as the vane may be helpful.

## 4 CONVERSION OF READINGS

The shear strength depends on the size of vane the instrument is used with. Multiply the reading of the scale-ring by the correct factor in the table below.

$$\text{Shear strength (kPa)} = \alpha \cdot \text{Reading}$$

Vane size in mm	Multiplicative factor $\alpha$
16 x 32	2
20 x 40	1
25.4 x 50.8	0.5

**Table 1: Factors to use for conversions**

*Note: If a correction on the shear strength should be applied because of friction on the shafts, do the correction on the reading before applying the vane coefficient by subtracting the torque friction. Please refer to the ASTM standard D2573 (volume 04.08) for more information.*

## 5 MAINTENANCE

The H-60 vane borer is simply designed, and does not require much attention. But it is most important to keep it as clean as possible. Periodically, the instrument should be sent back to factory for verification.

## 6 CONVERSION FACTORS

	To Convert From	To	Multiply By
LENGTH	Microns	Inches	3.94E-05
	Millimetres	Inches	0.0394
	Meters	Feet	3.2808
AREA	Square millimetres	Square inches	0.0016
	Square meters	Square feet	10.7643
VOLUME	Cubic centimetres	Cubic inches	0.06101
	Cubic meters	Cubic feet	35.3357
	Litres	U.S. gallon	0.26420
	Litres	Can-Br gallon	0.21997
MASS	Kilograms	Pounds	2.20459
	Kilograms	Short tons	0.00110
	Kilograms	Long tons	0.00098
FORCE	Newtons	Pounds-force	0.22482
	Newtons	Kilograms-force	0.10197
	Newtons	Kips	0.00023
PRESSURE AND STRESS	Kilopascals	Psi	0.14503
	Bars	Psi	14.4928
	Inches head of water*	Psi	0.03606
	Inches head of Hg	Psi	0.49116
	Pascal	Newton / square meter	1
	Kilopascals	Atmospheres	0.00987
	Kilopascals	Bars	0.01
	Kilopascals	Meters head of water*	0.10197
TEMPERATURE	Temp. in °F = (1.8 x Temp. in °C) + 32		
	Temp. in °C = (Temp. in °F – 32) / 1.8		

\* at 4 °C

E6TabConv-990505

**Table 2: Conversion factors**